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STUDIES OF LOW MOLECULAR WEIGHT LIGNIN SULFONATES

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Complete elemental and functional group compositions were established for lignin sulfonates from a spent sulfite liquor and compared to those from milled wood lignin preparation. This allowed an estimate of the degree of sulfonation, condensation and demethylation as well.

Extended separation studies indicated the low molecular weight lignin sulfonates to be the reaction product of a difunctional vinyl-type polymerization, thus accounting for the widely different properties as compared to their higher molecular weight counterparts. The feasibility of large scale separations was determined using (1) the extraction and precipitation of the dry matter in a spent sulfite liquor with alcohol, and (2) the fractionation of the material by ion exclusion in a column arrangement.

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STUDIES OF

LOW MOLECULAR WEIGHT LIGNIN SULFONATES

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ABSTRACT

Low molecular weight lignin sulfonates have been separated in purified form and characterized by physicochemical and chemical methods. Their structures and reactions have been evaluated. Preliminary studies on the feasibility of industrial scale separations have been performed.

Lignin sulfonates from the spent sulfite liquor of a mild acid bisulfite cook of Western Hemlock (Tsuga heterophylla) were purified and fractionated in Sephadex G-25 column chromatography. Samples with varying molecular weights were analyzed using a new method which aided the elimination of the polydisperse nature of the material under investigation. This method which involved acetylation of hydroxyl-groups and esterification of sulfonate-groups was widely developed in the course of this study using model compounds.

Complete elemental and functional group compositions were established for lignin sulfonates from a spent sulfite liquor and compared to those from milled wood lignin preparation, allowed an estimate of the degree of sulfonation, condensation and demethylation as well.

Extended separation studies indicated the low molecular weight lignin sulfonates to be the reaction product of a difunctional vinyl-type polymerization, thus accounting for the widely different properties as compared to their higher molecular weight counterparts (two- versus three-dimensional network).

The feasibility of large scale separations was piloted employing two different separation principles: one was the extraction and/or precipitation of the dry matter in a spent sulfite liquor with alcohol, the

second was the fractionation of the material by ion exclusion in a column arrangement. Both methods seem to allow the separation of basically three different classes of substances, namely, high molecular weight LS, low molecular weight LS, and carbohydrate degradation products.

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SECTION I

CONCLUSIONS

Low molecular weight lignin sulfonates can be separated in purified form from most other materials in spent sulfite liquors of the acid bisulfite pulping process by use of ion exclusion processes functioning in packed column equipment. Selective extraction or precipitation with alcohol of lignin sulfonates in aqueous solution does not yield particularly useful results.

Separation of purified lignin sulfonates into fractions of differing average molecular weights can be conducted effectively on a laboratory basis in aqueous solution by use of gel permeation with molecular sieve materials such as Sephadex 25 contained in a column.

The separated lignin sulfonates from two separate sources (one from a commercial spent sulfite liquor, and the other from a sulfonated milled wood lignin) showed gel chromatography elution patterns, ultra violet absorptions and certain other characteristics which were quite similar to each other in view of the differences in the two preparation procedures.

It was concluded that further useful characterization of the lignin sulfonate fraction might be accomplished by using a derivative soluble in organic solvents and therefore a procedure was developed for preparation of acetylated lignin sulfonate methyl esters (ALSME).

To provide experience toward synthesizing the ALSME preparations, acetylated methyl sulfonate ester derivatives were prepared for some eighteen different lignin sulfonate-like monomers and dimers. These were characterized by chemical analyses, NMR spectroscopy and mass spectroscopy.

Preparation of the ALSME fractions succeeded. These materials gave rise to NMR spectra which yielded further information concerning the structure of the lignin sulfonate molecules of differing molecular weights. Especially significant is the finding of an orderly change in phenolic hydroxyl content as a function of the molecular weight of lignin sulfonates.

Generally, the low molecular weight lignin sulfonates appear to arise perhaps mainly as hydrolysis products of high molecular weight lignin sulfonates, although some condensation or repolymerization of the low molecular weight entities seems to occur simultaneously.

SECTION II

RECOMMENDATIONS

The ion exclusion process for at least partial separation of lignin sulfonates from sugars and other materials in sulfite spent liquors should be developed further toward application on at least a small pilot plant industrial scale. The process desirably will be multi-stage and may be conducted intermittently or batchwise using a column packed with an appropriate ion exchange resin. Preferably the process should be carried out using continuous ion exclusion resion handling equipment as soon as such equipment becomes available.

Further laboratory studies should be carried out using the now-reported separation procedure for ALSME derivatives dissolved in organic solvents based upon use of an appropriate molecular sieve material such as Sephadex LH_{20}, and directed toward securing highly effective laboratory separations of lignin sulfonates of differing molecular weights. Simultaneously, further searches should be made to find organic solvents which dissolve lignin sulfonates and permit direct gel permeation chromatorgraphy separation. It seems possible that such procedures may permit the isolation in approximately pure form of lignin sulfonates with degree of polymerization up to five or possibly even up to ten.

Practially oriented research and development work should be carried out to learn how to make best economic use of the phenolic hydroxyl groupings indicated to be present rather extensively in low molecular weight lignin sulfonates. For example, a variety of ethers and esters could be produced which may have industrial uses.

The apparent capability of of the ALSME to polymerize under certain conditions suggests that further studies on the polymerizability of lignin sulfonates should be conducted, and upon the nature and possible commercial use of the resultant polymers.

SECTION III

INTRODUCTION

Lignin is a polymeric substance or class of substances, composed of phenol propane type units, which makes up about 20% to 30% of wood tissue. Throughout the world, wood cellulose or wood pulp is produced on a very large scale from woody tissue, leaving the lignins and lignin derivatives in spent pulping liquor which is usually evaporated and burned to recover heat and chemicals.

In one type of wood pulping process, the "sulfite" or "bisulfite process," delignification is accomplished by the use of aqueous solutions of metal bisulfites, either calcium, magnesium, sodium or ammonium bisulfite. Extensive investigations have been conducted and are continuing to be directed to the development of effective and economically competitive sulfite process recovery processes based on the recovery of effluent liquors, and then evaporating and burning to recover useful process chemicals and heat.

An alternative path is to develop methods for production of marketable by-products from the lignin. From the sulfite process, lignin sulfonates may be produced. Many studies have been conducted toward the objective of producing useful products from these materials and success in application and sales has been achieved in a few instances.

For further progress toward the aim of developing useful products, two main advances seem to be necessary, i.e., firstly, to secure more information concerning the physical nature and chemical structure and reactions of the lignin sulfonates and the 30% of substances which coexist

in the sulfite process effluent liquors, and secondly, to secure more information concerning how effectively and economically to separate these components one from another.

Toward these general objectives, research has been continuing in this laboratory over many years, and the studies now to be reported comprise a continuation of this effort.

Earlier Studies

Since about 1945, research on spent sulfite liquor and lignin sulfonates has been carried out at the University of Washington. During recent years this research has been concentrated on lignin sulfonates and the following specific work has been performed: Very low molecular weight lignin sulfonates, previously shown to comprise a few percent of the total lignin sulfonates of gymnosperms, have been fractionated using the techniques of column chromatography and multistage partition between butanol and water. Two monomeric lignin sulfonates and a dimeric lignin sulfonate have been isolated, crystallized, and shown to be the major components of the lowest molecular weight fractions. Evidence has been obtained for the presence of many other individual lignin sulfonates.

Dioxane lignin was prepared from carefully extracted wood and sulfonated. The resulting lignin sulfonates were separated into a high and low molecular weight fraction, using a new and fast method based on the solubility of low molecular weight sulfonates in n-butanol saturated with water. Upon chromatographic separation of the low molecular weight material and purification of the appropriate fractions, crystalline material was isolated and found to be identical with low molecular weight lignin sulfonates previously isolated from spent sulfite liquor.

The two monomeric lignin sulfonates were isolated, crystallized and identified as (3-methoxy-4-hydroxyphenyl)-2-propene-1-sulfonate and 1-(3-methoxy-4-hydroxyphenyl)-1-propene-3-sulfonate. The identity has been verified by synthesis (1, 2).

Recent Studies

In more recent work, sodium lignin sulfonates from gymnosperm woods were fractionated using Sephadex G-50 gel columns and eluting with distilled water or with 10^{-1} to 10^{-4} M NaCl solutions. Molecular weights of certain lignin sulfonate fractions were estimated by ultracentrifuge sedimentation equilibrium and were found to range in an orderly manner from about 70,000 in the mostly excluded initial eluates down to several hundred in the final eluates. The sizes of the lignin sulfonate polymer molecules in terms of equivalent Einstein spheres, r_η, were estimated from the measured molecular weights and from intrinsic viscosities calculated using relationships reported by Goring and coworkers. These ranged from about 7 to 70 Å (3, 4).

For a lignin sulfonate of a particular molecular weight, r_η was estimated to be up to two times greater in water than in 0.1 M NaCl solution, behavior expected for an elastic network containing negative charge sites in an environment of varying ionic strength.

We have shown that the concentration of sodium chloride in the aqueous eluant exerts a significant influence upon the degree of success attained in the fractionation of lignin sulfonates. To secure satisfactory reproducibility in fractionation, the concentration of electrolyte must be held constant. To secure optimum fractionation of lignin sulfonates, a particular level of eluant electrolyte concentration should be maintained which is appropriate in relation to the pore size of the molecular sieve material which is to be used.

These relationships are of particular significance because our research confirms the concept that the effective size of a lignin sulfonate molecule may be changed substantially by change in the concentration of electrolyte solution in which the molecule is dissolved.

When the size of the lignin sulfonate molecules falls approximately within the range of the sizes of the pores in the particular Sephadex molecular sieve, effective fractionation may occur. In further gel chromatography fractionations, gymnosperm lignin sulfonates in aqueous NaCl at 0.001 M and other molarities have been separated into ten substances or mixtures of substances amounting to about eighteen percent of the weight of total lignin sulfonates. The ultraviolet spectra of these was about the same as that of the unfractionated sample. Sedimentation equilibrium molecular weights of the entities ranged from several hundred up to about three thousand. The entities appear to be the lower molecular weight members of the polymer series of lignin sulfonates.

Based on the molecular weights measured for these "peak" substances, we have speculated that the peaks represent lignin sulfonate mers extending up to the 10-mer or 12-mer, and possibly the 14-mer (4).

SECTION IV

OBJECTIVES AND PLANS

Our general objective was to find new information concerning lignin sulfonates, and especially those of low molecular weight, which would provide a better foundation for development of profitable utilization or the innocuous and economic disposal of these materials, and of the spent sulfite liquors in which they are contained.

The specific aims of the research program were:

(a) To secure further knowledge of how to purify low molecular weight lignin sulfonates by separating them from other classes of materials, such as higher molecular weight lignin sulfonates, sugars and carbohydrates, inroganic substances, etc.

(b) To learn how to separate purified lignin sulfonates into fractions of differing molecular weights with certain factions hopefully to consist mainly of particular lignin sulfonate mers, such as monomers, dimers, trimers, and higher mers. It was anticipated that some crystalline monomers might be obtained but mainly it was expected that a mixture of several different mers would be found in each fraction.

(c) To characterize each lignin sulfonate fraction by a number of spectroscopic and other available procedures in order to secure additional knowledge of the chemical structure and reactions of lignin sulfonates.

(d) To prepare and characterize derivatives of low molecular weight lignin sulfonates to augment the information gained by characterization of the lignin sulfonates themselves.

(e) To try to find new approaches to the economic utilization of lignin sulfonates.

SECTION V

PREPARATION, FRACTIONATION, AND PRELIMINARY CHARACTERIZATION OF GYMNOSPERM LIGNIN SULFONATES

Introduction

The first step taken to initiate the investigation was to proceed with the preparation of the gymnosperms lignin sulfonates (for prior work, see ref. 5,6,7). In view of the importance in the pulp and paper industry, Western Hemlock wood was chosen as the basis for the investigation.

Purification and Separation

By courtesy of the Weyerhaeuser Company in Everett, Washington, spent sulfite liquor was prepared by pulping Western Hemlock in a laboratory digester under mild conditions utilizing calcium base pulping liquor. Mild conditions were used in order to secure a larger than usual proportion of low molecular weight lignin sulfonates in the resultant calcium base sulfite liquor.

This calcium base liquor was then treated in accordance with the flow sheet given as Figure I; and then, more specifically, according to the following procedure. The liquor was degassed, passed through Dowex 50-8X to remove metal ions and yield free acids, then neutralized with barium hydroxide. The barium sulfate precipitate was removed by centrifuging and the final material concentrated by evaporation.

A sample of this concentrated BaSSL was applied to a column containing Dowex 50W-X2 ion exchange resin to achieve separation of strong electrolytes (lignin sulfonates) from weak electrolytes (acetic acid, aldonic acids) and non-electrolytes especially including sugars. The effluent was collected intermittently after having been examined by a

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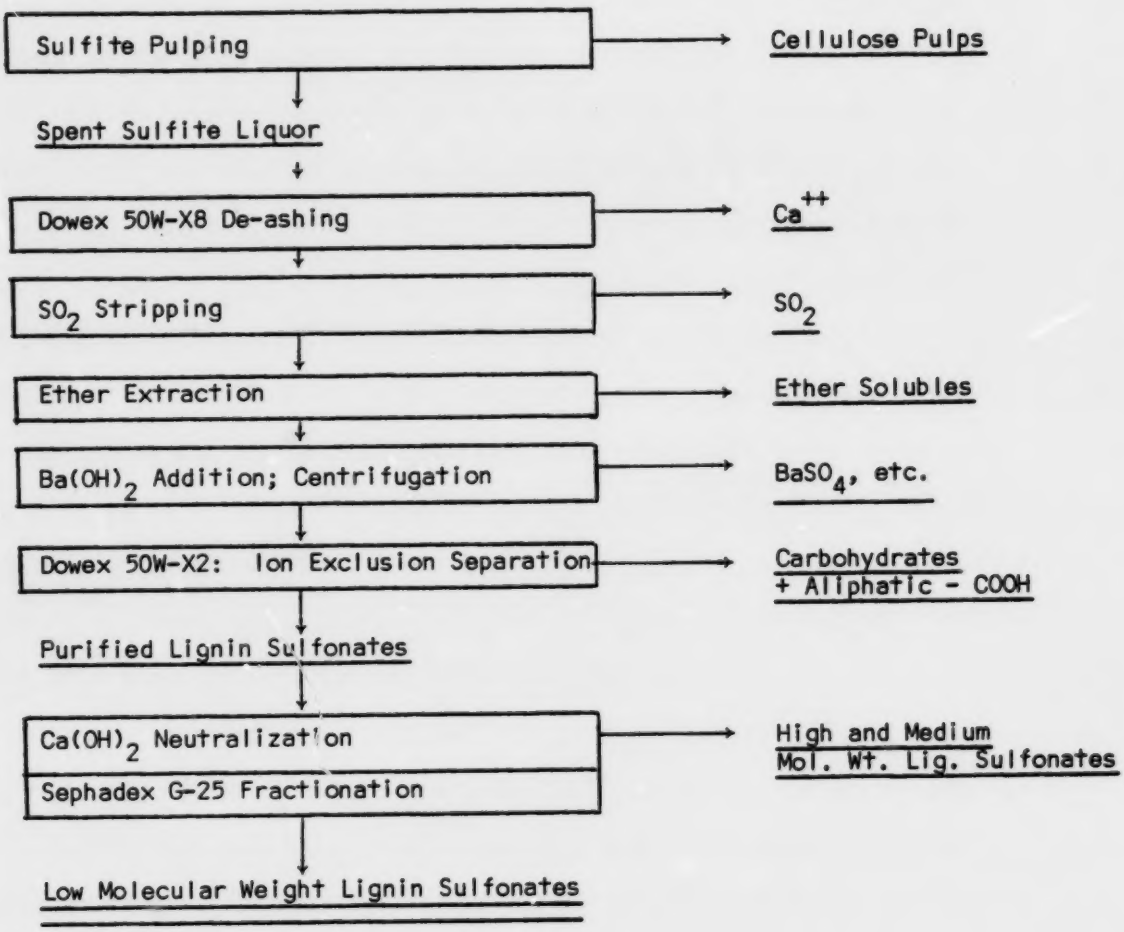
FIGURE I

Preparation of Spent Sulfite Liquor



continuous UV recording apparatus, then neutralized with calcium hydroxide and concentrated by evaporation. This CaSSL was subjected to Sephadex G-25 gel chromatography and the absorbance at 280 nm of the resultant elution liquid was recorded and is shown in Figure 2.

The relatively small size of the peak in the effluent volume range 2,200 - 2,600 ml in Figure 2 shows that the spent sulfite liquor contained only a small proportion of lignosulfonates excluded by Sephadex G-25, i.e. lignosulfonates with molecular weights larger than 10,000 (7). This is in all probability due to incomplete delignification during the cook that was performed in order to produce a high proportion of low-molecular-weight sulfonates.

The eluted liquid was divided into a series of sixteen fractions (A-P) and in each case, an overlapping peak was defined by thin layer chromatography using isopropanol-water developer. The mid-section of corresponding peaks for a series of runs were combined and freeze dried. The resultant material was subjected to analysis by ultraviolet and infra red spectroscopy.

Characterization by UV-Spectroscopy

Ultraviolet absorption spectra of a selection of fractions marked A - P in Figure 2 were recorded on a Cary registering spectrophotometer in neutral and alkaline solution. The overall shape of the spectra obtained is well known to lignin chemists and therefore only the wavelengths of the absorption maxima and minima are shown in Table I.

As expected, all fractions A - P show in neutral solution an absorption maximum near 280 nm. A closer examination reveals, however, that fractions A - C exhibit the maximum at 281 nm whereas the maximum in

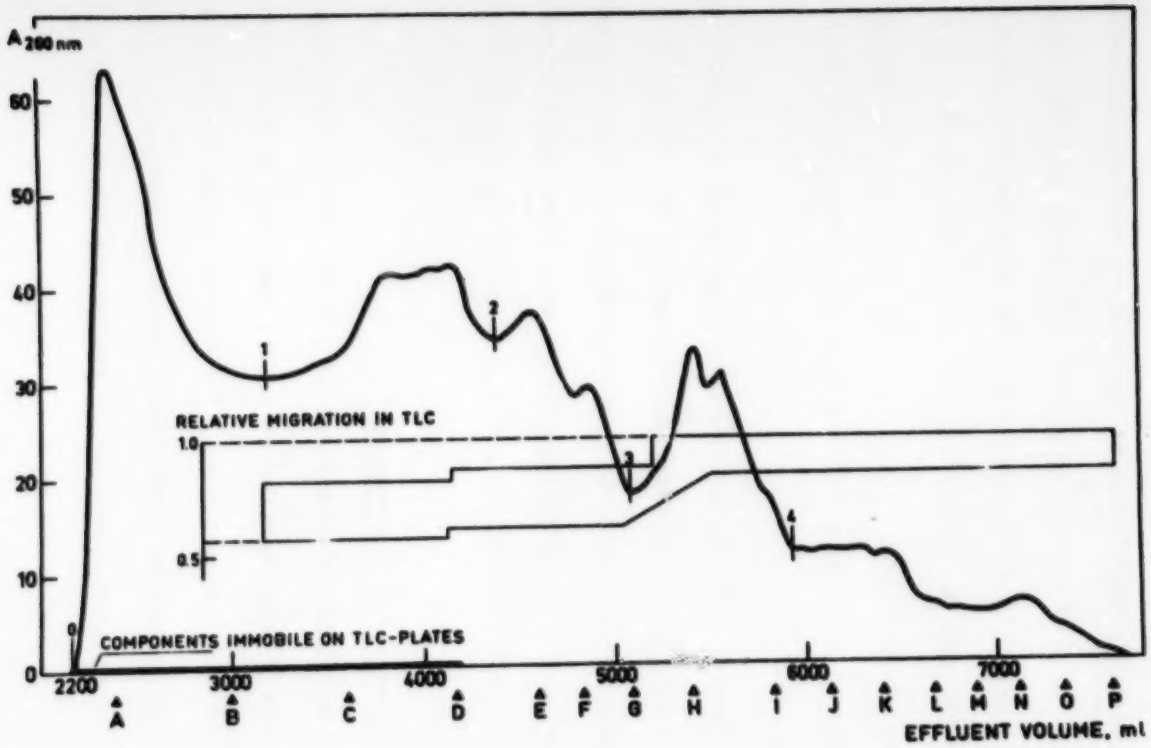


FIGURE 2.

Fractionation of Sulfonates in Spent Sulfite Liquor by Gel Chromatography on Sephadex G-25 in a Column 10 feet by 2 inches ID with Water as Eluant.

fractions D - K is found at 280 nm and in fractions L - P at 281-282 nm. This very small difference in the location of the absorption maximum can also be seen in the spectra of the fractions of sulfonated milled-wood lignin.

In neutral solution fractions A, B and C of both spent sulfite liquor and milled wood lignin show an absorption minimum at 260-261 nm whereas fractions D, E and G show a minimum at 258 - 259 nm. Fraction I shows a minimum at 256 - 257 nm.

In alkaline solution, fractions A, B and C show a maximum at 282 - 283 nm, fractions D, E and F at 285 - 286 nm. Fractions G - K show maxima in the region 287 - 293 nm with the maximum shifting towards longer wavelengths with increasing effluent volume (and probably decreasing molecular weights). Fractions L - P show the absorption maximum at 298 - 299 nm.

A division of the fractions of Figure 2 into the four groups I - IV can be done on basis of the shift of the absorption maximum near 280 nm on alkalizing the solution as can be seen from Figure 3.

Figure 3 shows that fractions A - C of the spent sulfite liquor showed a very small red-shift of only 1 - 2 nm. Fractions D - F showed a red-shift of 5 - 6 nm, fractions G - K showed a red-shift of 8 - 13 nm and fractions L - P a shift of 16 - 17 nm.

Characterization by Thin Layer Chromatography

About every third 10 ml fraction of Figure 2 was further studied by means of thin layer chromatography (TLC) on analytical silica gel G ("Merck") plates using 4:1 isopropanol-water as eluent. The spots were visualized by illuminating with ultraviolet light.

TABLE I

CHARACTERISTICS OF ULTRAVIOLET ABSORPTION SPECTRA OF FRACTIONS A - P IN FIGURE 2

Fraction	Neutral Solution		Alkaline Solution
	Maximum	Minimum	Maximum
A	281	261	282
B	281	261	282
C	281	260	282
D	280	259	286
E	280	258	286
F	280	258	286
G	280	258	288
H	280	258	290
I	280	257	291
J	280	256	293
K	280	256	293
L	281	261	298
M	282	262	299
N	282	262	299
O	282	262	299
P	282	260	298

These studies showed that the fractions up to an effluent volume of 3,200 ml contained only solutes immobile on the TLC-plates. The fractions in the range 3,200 - 4,100 ml contained immobile as well as mobile components. In the fraction range 4,100 - 7,600 ml the fractions contained only mobile components.

Each fraction containing mobile components formed two or three spots on the TLC-plates. The location of these spots is indicated in Figure 2. A comparison with Figure 3 shows that the immobile solutes are to be found almost completely in fraction group I that contains only small amounts of

mobile solutes. The mobile solutes are mainly to be found in fraction groups II - IV.

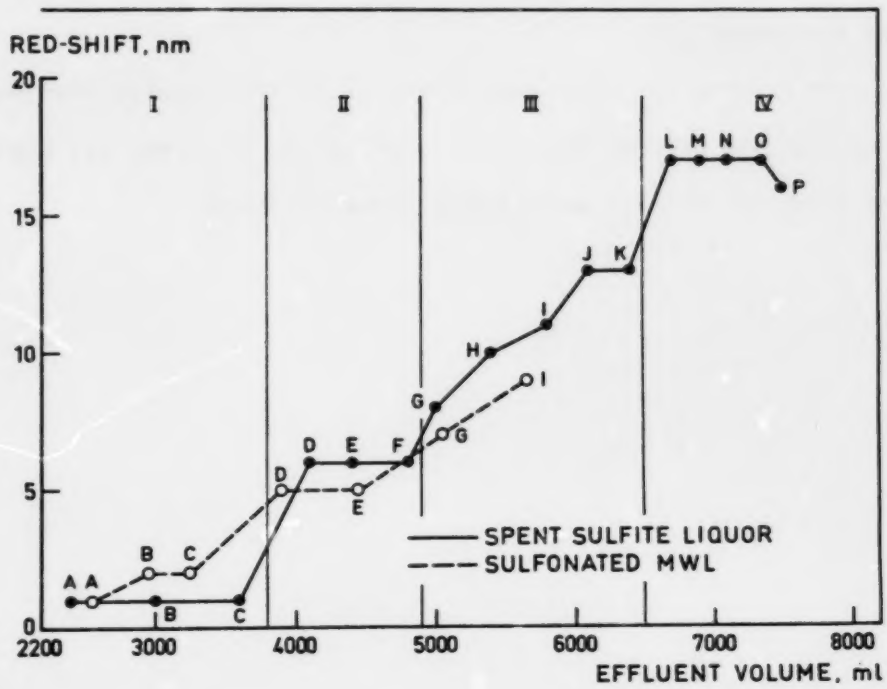


FIGURE 3.

Shift of the Wavelength of the Absorption Maximum Near 280 nm on Alkalizing Fractions of the Spent Sulfite Liquor and of the Sulfonated Milled Wood Lignin Shown in Fig. 2.

Conclusions

From the presented results, it can be concluded that there are distinct differences between high- and low-, as well as between low- and low molecular wight lignin sulfonates. These differences are revealed by gel permeation as well as thin layer chromatography and UV-spectroscopy. Classification into four different groups (I-IV) seemed to be approximate.

A substantial red-shift was evidenced upon alkalination for the lowest molecular weight lignin sulfonates, which indicates the presence of some unique functional groups, probably in the side chains of the C_9-units of the sulfonates.

An examination of various fractions by TLC revealed that the sulfonates could be divided into those immobile on TLC plates and those mobile. The immobile sulfonic acids formed brown solutions.

SECTION VI

PREPARATION OF CERTAIN LOMOLS-LIKE MONOMERS AND DIMERS AND CHARACTERIZATION BY NMR AND MASS SPECTROGRAPHY

Introduction

Preliminary investigation of the fractions secured from the Sephadex separations, as described in Section V of this report, indicated the need for application of the more refined analytical methods that are nowadays commonly used throughout organic chemistry. Whereas, NMR- and Mass-spectroscopy had been successfully applied to study lignin and lignin-like derivatives (8 - 12), these common methods could not hitherto be used for lignosulfonates.

However, to apply these procedures, it was necessary to secure information concerning the spectra or patterns associated with particular groupings expected to be present in the LOMOL type materials. It thereupon became necessary to assemble and/or synthesize certain LOMOLS like monomers and dimers in order to permit the spectroscopy to be carried out in consideration of the special chemical groupings of interest.

In contrast to other organic natural compounds, the analytical basis for the characterization of lignin sulfonates remained widely restricted because of the polyelectrolytic and polydisperse nature of the material. The full elucidation of the side chain structures and types of linkages has not been achieved, in part because available analytical methods have not provided sufficient information. Due to the insolubility of lignin sulfonates in organic solvents and their thermal instability, however, methods such as NMR- and mass spectroscopy, extensively used in structural studies of organic natural compounds, could not be applied. Our studies,

therefore, were directed to establish modification procedures which allow the conversion of lignin sulfonate fractions into derivatives with the desired physical and structural properties by covering the polar groups such as hydroxyl- and sulfonic acid groups without evoking changes of the original side chain structures.

Another requirement of the transformation procedures to be applied is their applicability to all the various lignin structures over the entire range of molecular weights. To cover both the phenolic- and the alcoholic hydroxyl groups as well, we applied the acetylation by pyridine/acetanhydride.

To cover the sulfonic acid groups, the conversion to derivatives such as acid chlorides, acid amides and esters was considered (13). Previous attempts to characterize acetylated monomeric lignin-type sulfochlorides by mass spectroscopy, however, gave unsatisfactory results (14). As a consequence, we explored the possibility of establishing a suitable method for esterifying the rather labile and reactive sulfonic acid groups dominantly located in the α-position (benzyl-sulfonic acids) of lignin side chains.

Synthesis

A variety of lignin related model compounds featuring pertinent side chain structures and type of linkages were synthesized and sulfonated. By following the briefly outlined transformation procedure (Figure 4) the corresponding acetylated methyl sulfonates were formed (15, 16). The obtained derivatives were soluble in organic solvents and purified by recrystallization and exhibited the physical properties required for NMR- and mass spectroscopic analysis.

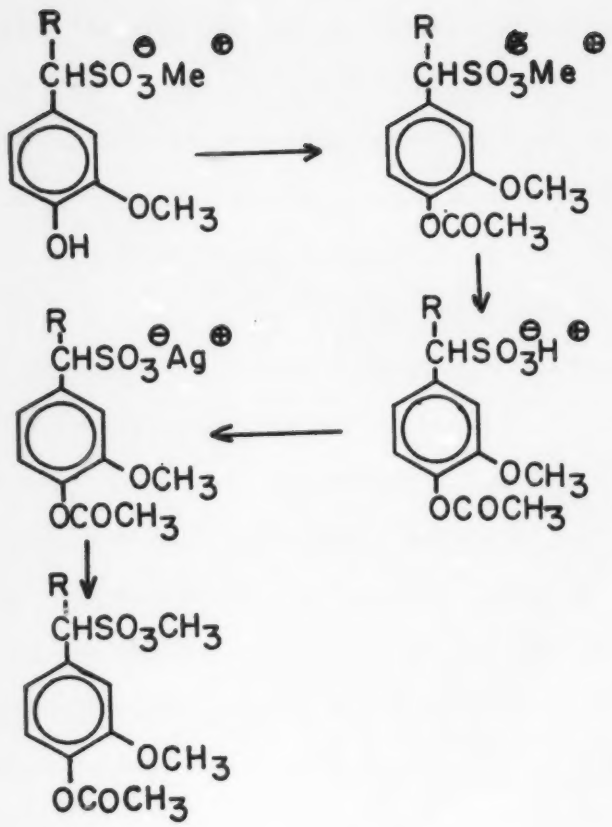


FIGURE 4.

REACTION SCHEME FOR THE TRANSFORMATION PROCEDURE.

As shown in Figure 4, sodium sulfonates were acetylated by pyridine/ acetanhydride. The aqueous solution of the free acids prepared by cation exchange was neutralized by silver oxide. Reaction with methyl iodide yielded the corresponding acetylated methyl sulfonates.

The 18 model compounds that were synthesized in the course of this study are represented by four types of monomeric and dimeric lignin building units, as shown in Figure 5.

Type 2 represents monomers of the guaiacyl- and veratryl- series with saturated and unsaturated sidechains. It includes all three possible reaction products of the coniferylalcohol-type structure. Among them is one α - and one γ- sulfonic acid, each with a double bond, and one α- γ - disulfonic acid. All of the three types of sulfonic acids have been isolated earlier from sulfite liquors (1, 2).

The second type of linkage is expected to represent the major portion of linkages present in lignin. The most important one of them is the configuration which carries an aryl-ether structure in β-position and a hydroxyl group in γ-position. Other substituents to consider at the β-carbon atom are a hydroxylgroup or a carbon-to-carbon linked second C_9 unit as it is formed from the coumaran structures in lignin in a neutral sulfite cook. A compound with the latter configuration was supplied to us by Dr. Josef Gierer from Sweden. The fourth type of linkage represents the diphenylstructure.

NMR-Spectroscopy

Extensive studies performed earlier in this laboratory (8, 9) revealed that NMR-spectroscopy represents a valuable method to study the overall structure of different lignin preparations. However, the NMR

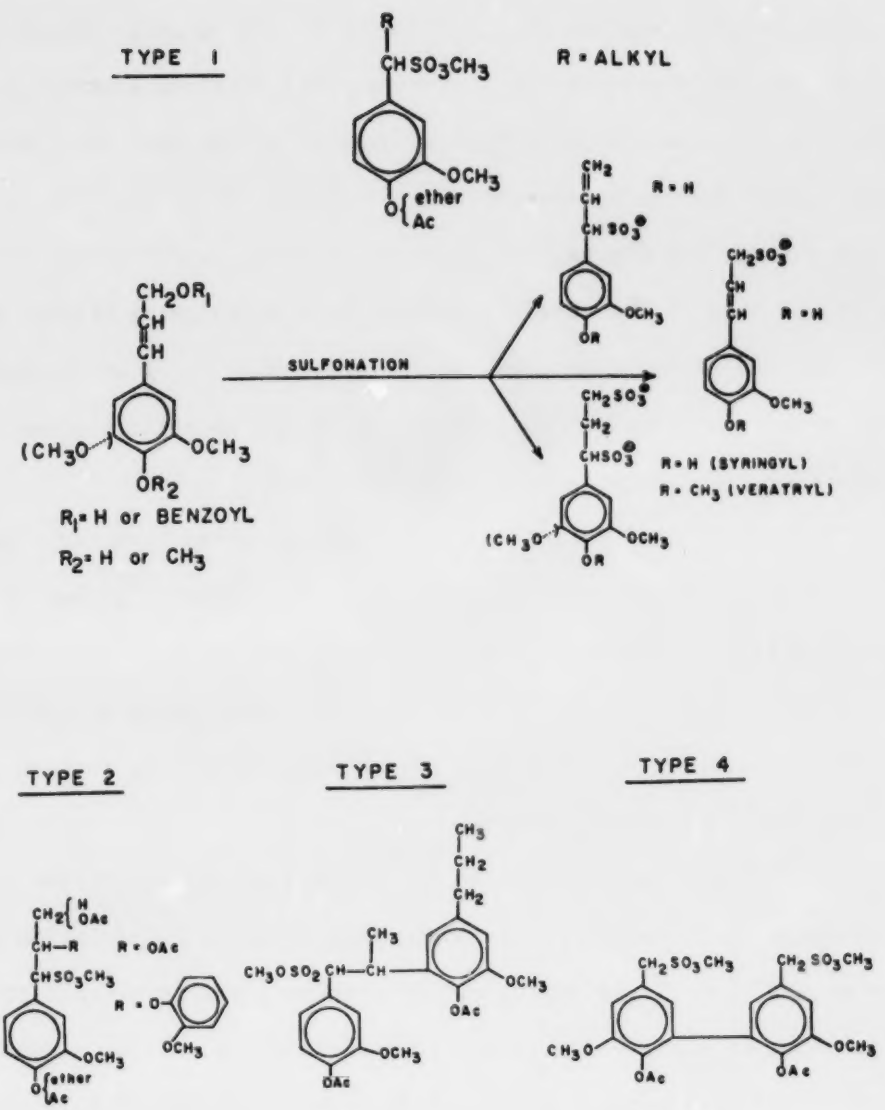


FIGURE 5.

THE FOUR TYPES OF LOMOLS-LIKE MONOMERS AND DIMERS SYNTHESIZED.

spectra of sulfonated lignin models and lignin sulfonate fractions did not yield satisfactory information mainly due to the lack of solubility in organic solvents and due to the presence of strong polar groups. By preparing the acetylated methyl sulfonates well resolved spectra could be obtained. Figure 6 shows a typical example of the NMR-spectrum of a sulfonated dimer. Following the increasing magnetic field from the left to the right side of the Figure, the aromatic protons are exhibited first, followed by the protons of the benzylic carbon atoms which carry acetoxy-groups. Methine- and methylene-protons of the sidechains of C_9 units generally give rise to signals between the region of the aromatic protons and the protons of the methoxylgroups, with which they interfere.

Protons of acetylgroups are much less effected by other signals than are methoxy-protons. Aromatic acetoxy-protons usually appear at lower field, well separated from their aliphatic counterparts; however, biphenyl and phenyl-alkyl linkages ortho to acetoxy-groups cause a significant shift of the acetyl-protons to higher field, where they interfere with the aliphatic acetyl-groups.

The average chemical shifts for acetylated and esterified lignin sulfonates in NMR-spectroscopy are listed in Table 2. This Table provides the possibility of estimating rather precisely the relative distribution of protons in LOMOLS-like materials and therefore, allows a nearly complete functional group analysis of the acetylated methyl sulfonates.

Mass Spectroscopy

Systematical mass spectroscopic studies of lignin related models had not been reported before we started with our investigations. However, several papers dealing with the mass spectroscopic fragmentations patterns

TABLE 2

CHEMICAL SHIFTS FOR ACETYLATED AND ESTERIFIED LIGNIN SULFONATES IN NMR SPECTROSCOPY (τ-VALUES)

Region	Location of Signals	Type of Protons
2	2.00 - 3.72	aromatic H, except in sulfonated coumarans, α-vinyl-protons
3, 4	3.72 - 4.82	aromatic H in sulfonated coumarans, β-vinyl-protons partially $C_\beta H$ $C_\alpha H$-O-Ac (non-sulfonated) $C_\alpha H$-O-Ar (in coumarans)
5	4.82 - 7.50	Methoxyl-protons, sidechain protons other than in 3, 4 and 8
6	7.50 - 7.81	Aromatic acetyl-protons, except those ortho to biphenyl-linkages
7	7.81 - 8.42	Aliphatic acetyl-protons, aromatic acetyl-protons ortho to biphenyl-linkages
8	8.42 - 9.62	highly shielded, mostly sidechain-protons

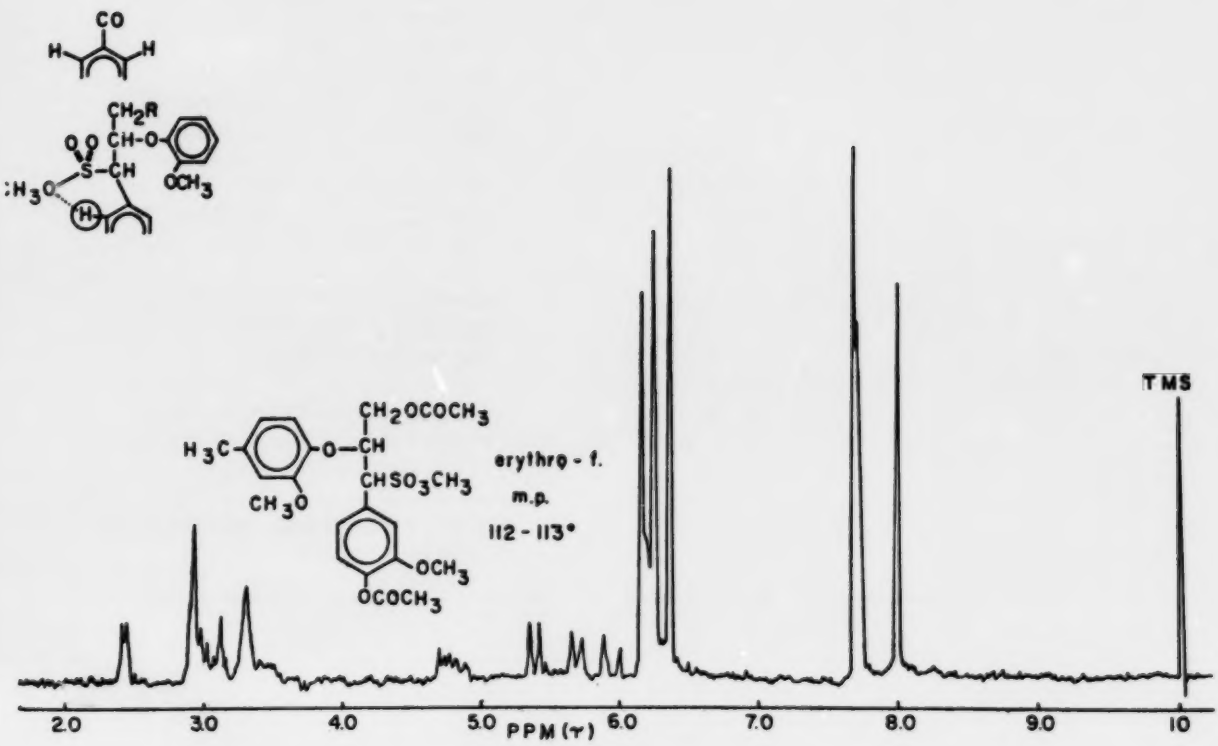


FIGURE 6.

NMR-SPECTRUM OF A TYPICAL LOMOLS-LIKE DIMER.

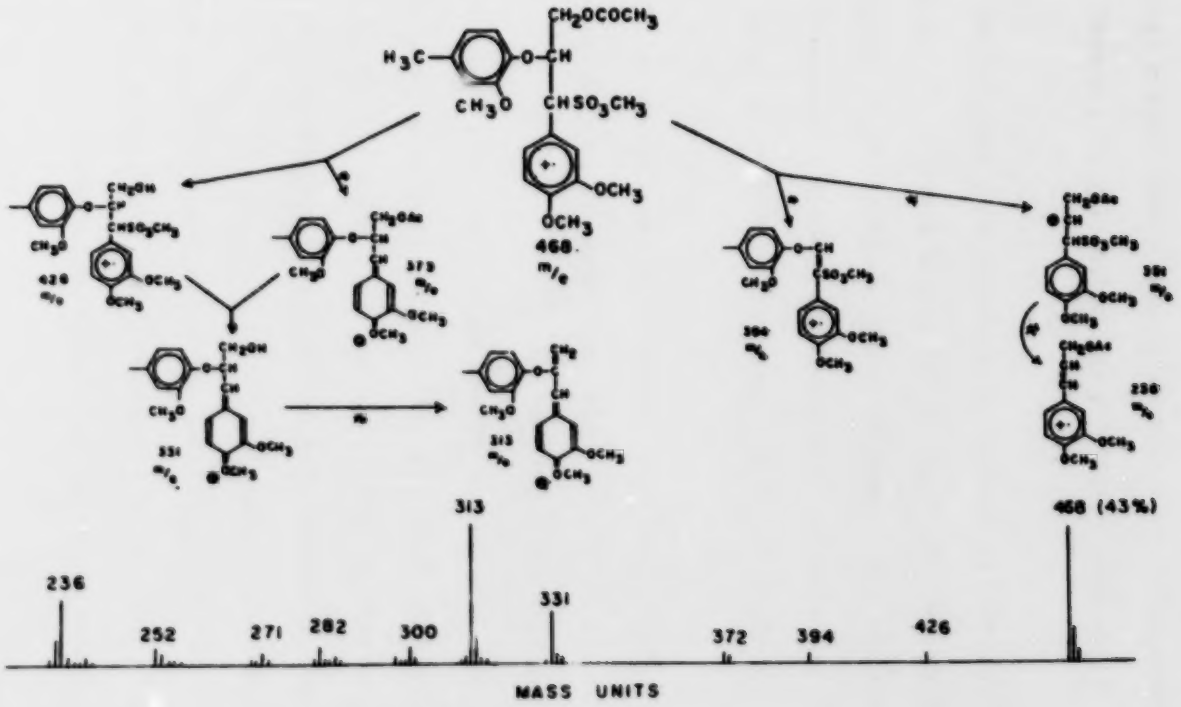


FIGURE 7.

MASS-SPECTRUM AND FRAGMENTATION PATTERN OF A TYPICAL LOMOLS-LIKE DIMER.

of rather simple models have been reported (11, 12). Earlier attempts, however, to obtain useful data from sulfonated lignin models failed because of either the poor volatility or instability at elevated temperatures.

The acetylated methyl sulfonates, however, were found to be volatile and thermally stable enough to yield reasonable spectra. Although the base peaks were quite low in several cases, exact molecular weights could be obtained. In our studies we recorded the spectra of monomeric and dimeric models. A characteristic fragmentation pattern is shown in Figure 7.

Conclusions

Sulfonation studies with lignin model substances provided us with some information about the principal reaction pathway of the chemical reaction that occurs in the sulfite pulping of wood.

The highly desirable use of modern analytical tools in organic chemistry was made possible through a new transformation procedure for sulfonated lignin-like materials, which involved acetylation and esterification. This method allowed transformation of the lignin sulfonates into derivatives without electrolytic behavior.

With the aid of such acetylated and esterified lignin sulfonate model compounds the analytical basis for NMR and Mass spectroscopy studies with lignin sulfonates from spent sulfite liquor was created.

SECTION VII

PREPARATION OF ACETYL LIGNIN SULFONATE METHYLESTER (ALSME) AND THEIR SPECTROSCOPIC CHARACTERIZATIONS

Introduction

The polyelectrolytic nature of lignin sulfonates has been the main obstacle to their fractionation and characterization in the past. To overcome the analytical difficulties which were caused by this property, we investigated the transformation of these compounds into derivatives with properties required for structural studies, especially for NMR- and mass spectroscopy. The synthetic basis for this transformation and the analytical basis for the characterization of such derivatives had been established before, using monomeric and dimeric lignin building units as models. (See Section VI for details.) The transformation aimed at the elimination of the polyelectric behavior by covering the polar groups: hydroxyl groups by acetylation, and sulfonic acid groups by esterification. Such acetylated lignin sulfonate methyl ester (hereafter called "ALSME") would be sufficiently soluble in organic solvents to allow the application of the more powerful analytical tools, such as NMR- and mass spectroscopy.

Transformation Procedure

The procedure used for the transformation of the LOMOLS-like monomers and dimers was applied to lignin sulfonates with varying molecular weights in a slightly modified form. The so-obtained ALSME products were found to be soluble in chloroform, the solvent commonly used for recording NMR-spectra.

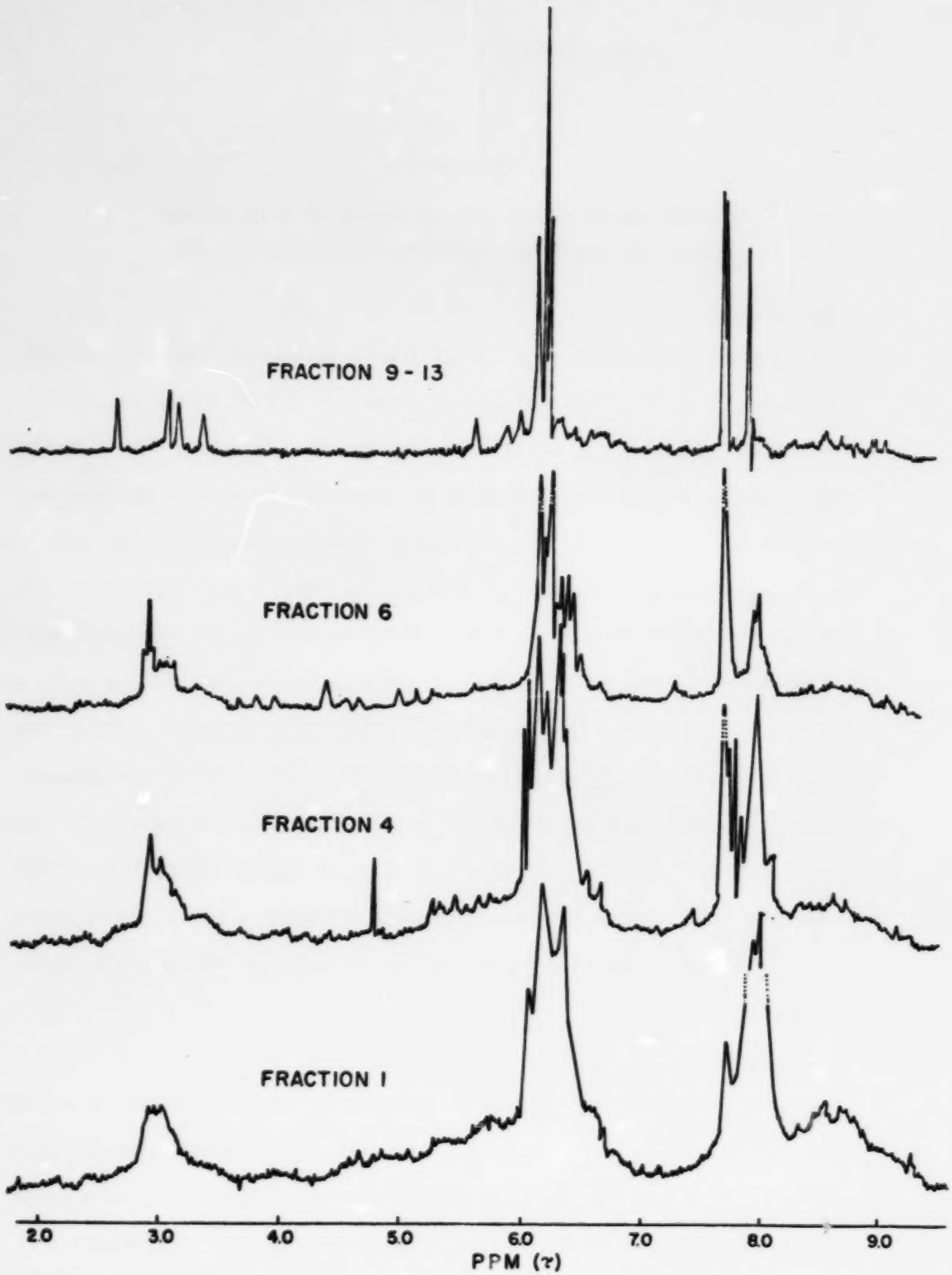


FIGURE 8.

NMR SPECTRA OF ALSME DERIVATIVES, PREPARED FROM VARIOUS FRACTIONS FROM SEPHADEX G-25 SEPARATIONS.

The transformation to ALSME derivatives resulted also in a purification of the original lignin sulfonate fractions obtained from the previously described Sephadex fractionation (See Section V), which were contaminated with carbohydrate degradation products to a certain degree.

NMR Spectroscopy: The spectra were recorded using the same conditions as described for the LOMOLS-like monomers and dimers (See Section VI). The entire frequency range of each spectrum was divided into several areas that were classified on the basis of the model studies. As shown in Figure 8, the LOMOLS exhibited well resolved spectra in a wide range of the investigated fractions.

Chromatography: The separation of ALSME fractions using the cross-linked Sephadex gel LH-20 and methanol as eluant resulted in the fractionation of low molecular weight mixtures into simple mixtures and into a single, crystalline compound (m. p. 200-201^{o}C).

Analytical thin-layer chromatographic studies and fractionations by Sephadex LH-20 gel chromatography in analytical scale indicate that the fractions are composed of more or less complex mixtures of low molecular weight compounds ranging from monomers to dimers and trimers and some higher mers (See Figure 9).

Characterization by NMR Spectroscopy

In general, the spectra of all thirteen fractions studied allowed a good correlation between the protons of various functional groups. Special emphasis was placed upon the content of aliphatic and phenolic hydroxyl groups. The correlation between these two hydroxyl fractions led finally to a classification of the fractions in four different groups. Constant ratios--aliphatic to phenolic hydroxyls--ranging from 0.5 (fractions I to 7

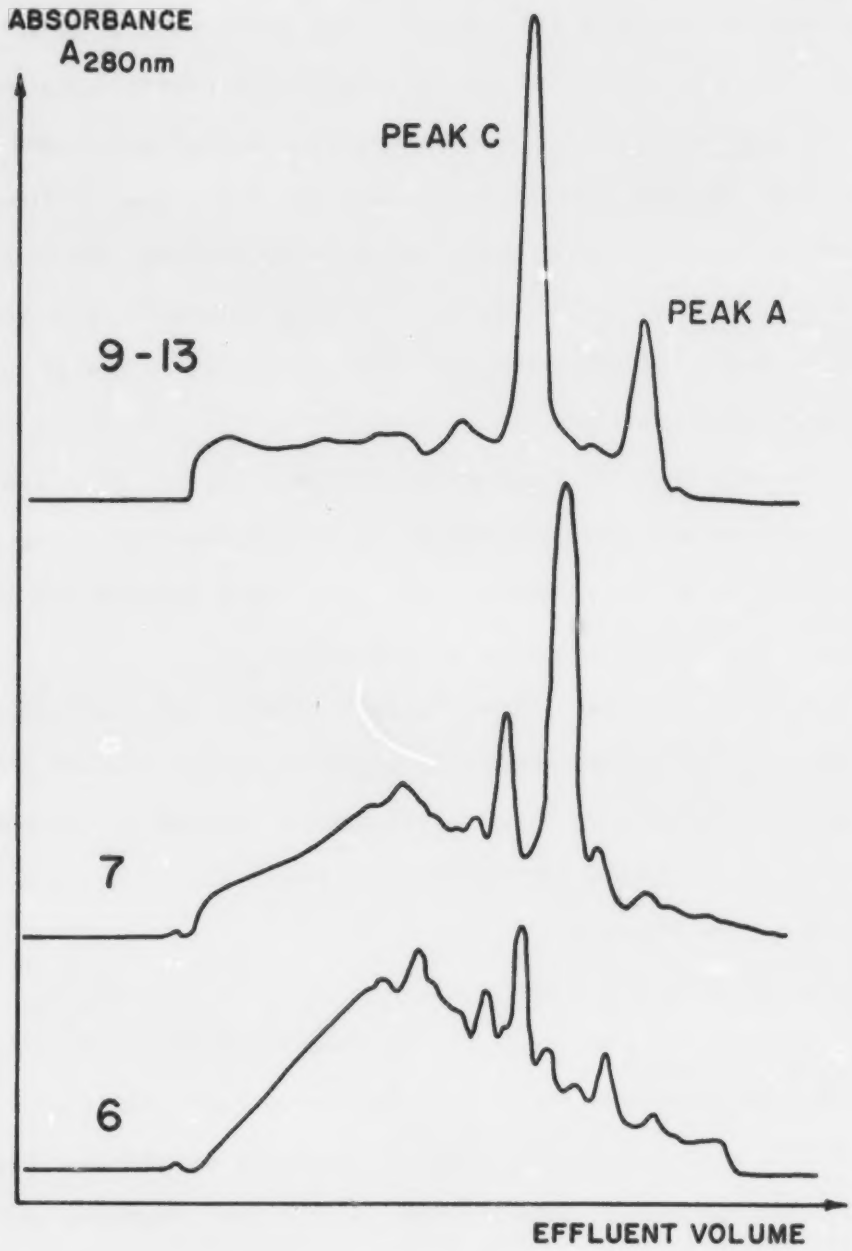


FIGURE 9.

SEPHADEX LH-20 SEPARATIONS OF SOME ALSME FRACTIONS IN METHANOL.

[Fractions 1-13 correspond with fractions A-P in Section V in reverse order]), 1.0 (fraction 8, 9), and 1.4 (fractions 10, 11) were observed (see Figure 10). In the next higher fractions we found a continuous increase of this ratio.

A similar classification in a somewhat different fashion could be made on the basis of the UV-spectra (See Section V). These findings were finally supported by the remaining NMR signals which could be assigned to protons of defined side chain structures. The main component in the group consisting of the lowest molecular fractions, fractions 1-5, (See Section V and Figure 2) was indicated but not established to be a dimer of a lignan-type structure.

The next higher fractions (6, 7) represent mixtures of different structural makeup.

The lignan-like compound was finally isolated over Sephadex LH-20 and purified by recrystallization. Its melting point, its NMR-spectrum, and its mass spectrum indicate that it is of dimeric structure. Its absolute configuration so far has not yet been established.

In fractions 8, 9 a vinyl sulfonic acid type side chain structure could be detected.

The overlapping and broadening of the signals found in the spectra of the next higher fractions does not allow further studies on side chain structures. However, we did find indications that condensation presumably in 5-position increases with the molecular weight. The first indication of condensation in this way is observed in fraction 9 and seems to increase steadily in the higher fractions. It remains unknown whether this condensation is induced secondarily by the sulfonation reaction, or whether it is present in the original lignin in the plant.

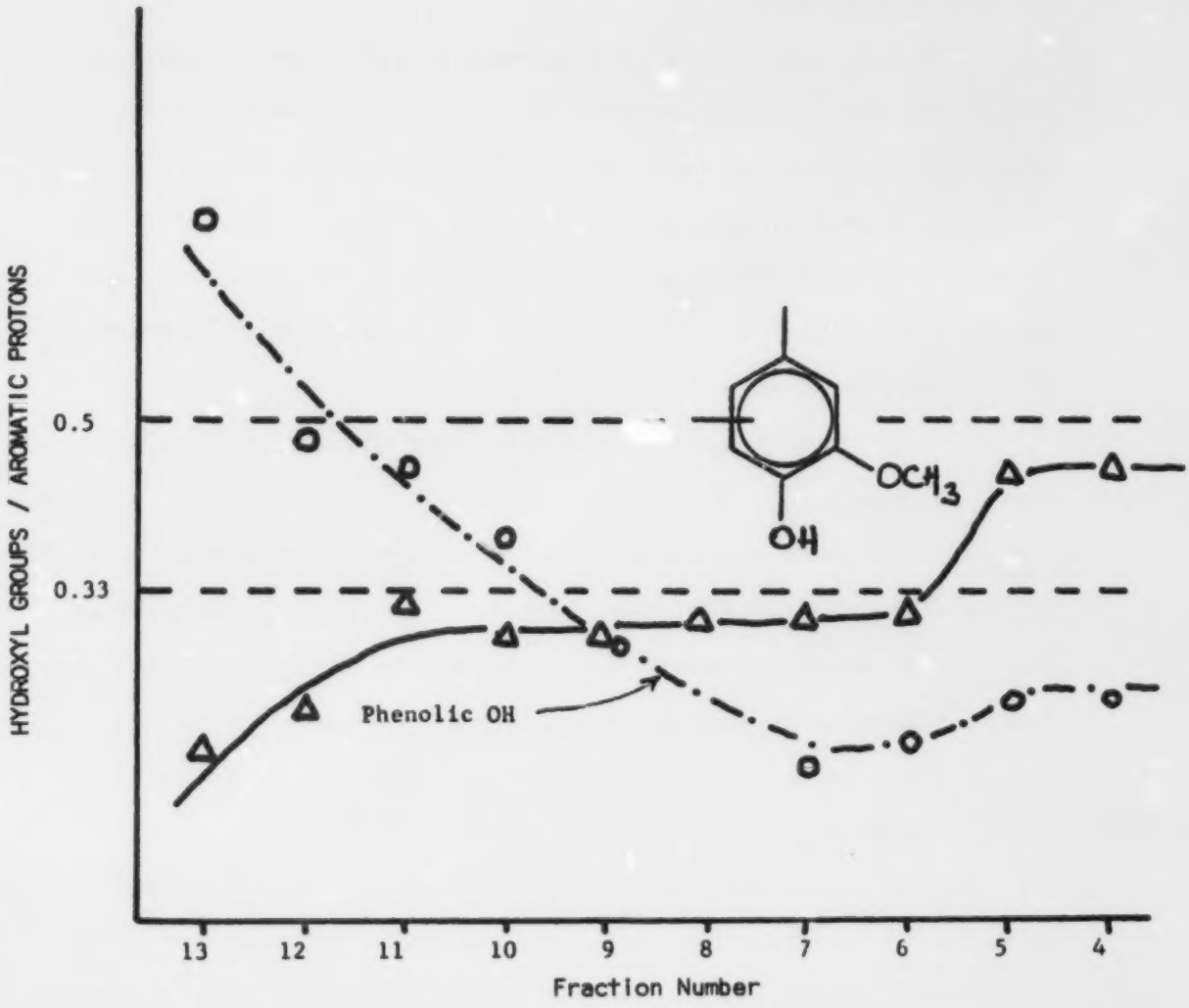


FIGURE 10.

CORRELATION BETWEEN PHENOLIC HYDROXYL GROUPS AND AROMATIC PROTONS IN FRACTIONS FROM SEPHADEX SEPARATIONS.

Characterization by IR Spectroscopy

The IR spectra of ALSME derivatives are all very similar and without characteristic features. The sulfonate group exhibits a strong band around 11 and 12 , which is rather untypical. The acetyl groups raise strong ester bands in the neighborhood of 5.8 , thus covering eventually present carbonyl groups. These carbonyl groups, however, can be detected in the IR spectra of the untransformed fractions. It could be shown that the carbonyl content of the LOMOLS is usually very low. Some fractions, however, in the very low molecular weight range exhibit a distinct concentration of CO-bands (See Figure 11). No attempts were made to investigate the nature of the carbonyl groups, which might also be due to incomplete separation from carbohydrate degradation products.

Conclusions

The polyelectrolytic and polydisperse nature of the LOMOLS could be eliminated by a relatively simple transformation procedure that covered the highly polar groups. We assume that this transformation did not induce any particular changes in the original structures of the LOMOLS. The so-obtained ALSME derivatives were found to be soluble in organic solvents, which aided considerably their analysis.

The most valuable analytical method proved to be NMR spectroscopy. It could be demonstrated that the NMR spectra of low molecular ALSME derivatives provide information on structures which could not be obtained from other analytical and spectroscopic methods so far. Indications on typical side chain structures and types of condensations can be as well derived as an almost complete functional group analysis. It could be demonstrated that the content of aliphatic and phenolic hydroxyl groups can be estimated

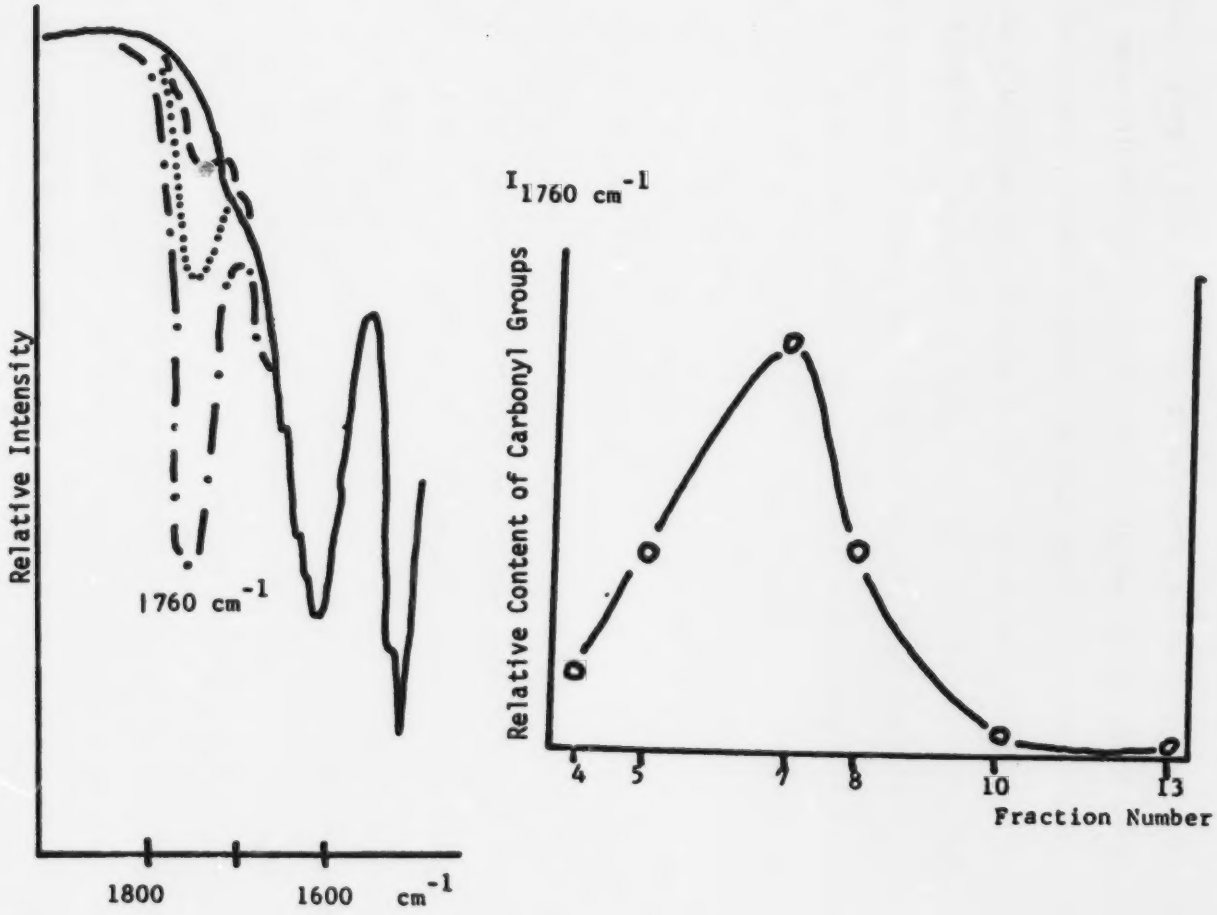


FIGURE 11.

THE CARBONYL REGION IN IR - SPECTRA OF FRACTIONS FROM SEPHADEX SEPARATIONS.

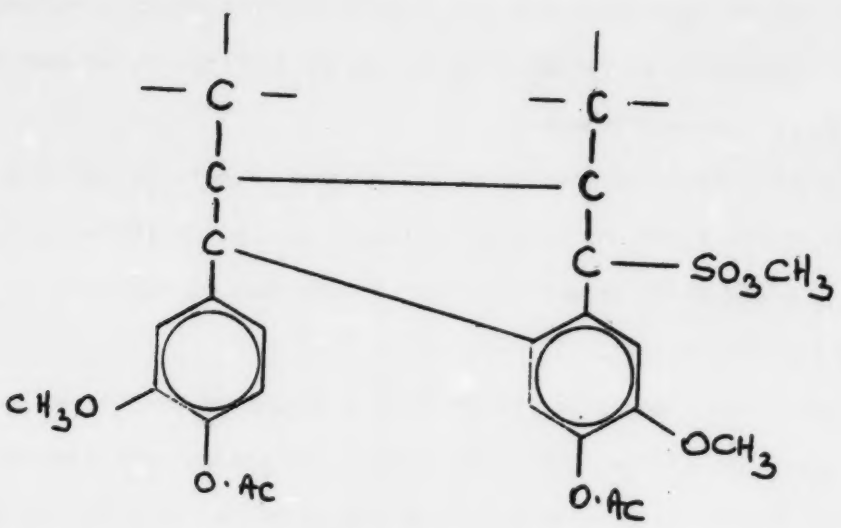
in higher molecular lignin sulfonate fractions with an accuracy never accomplished before, even by applying rather elaborate analytical techniques. The NMR spectroscopic technique represents the only method known in lignin chemistry so far which allows us to distinguish between these two types of hydroxyl groups.

The correlation between these two hydroxyl functions led to a classification of the fractions in four different groups. A similar classification in a somewhat different fashion could be made on the basis of the UV spectra (see Section V).

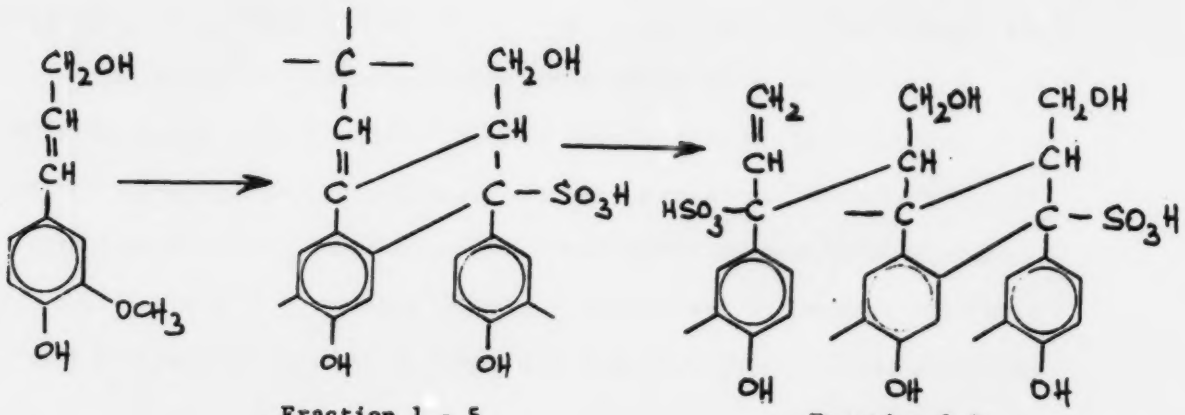
Significant changes in the structural makeup of the low molecular weight fractions of the ALSME were found. The predominant component in the first five fractions exhibits features characteristic for lignan-type structures. Since unextracted Western Hemlock was used for acid bisulfite pulping the presence of sulfonated lignans would not be surprising. Another explanation, however, seems to be that this dimer is formed by a vinyl-type condensation of two monomers with conjugated double bonds. Based on the assumption that coniferyl alcohol is the only precursor, our findings can be interpreted as depicted in the reaction scheme in Figure 12.

Homocondensation of coniferyl alcohol forms a lignan-type dimer with the structure that represents the main compound in fractions 1-5. Further condensation between the dimer and coniferyl alcohol and subsequent allyl type rearrangement of the coniferyl alcohol side chain to a vinyl-type structure leads to a product with the spectral features observed in fractions 8 and 9.

Although there is considerable doubt about the validity of the proposed condensation mechanism, this hypothesis could very well explain some of the observed discrepancies of LOMOLS. Further experiments are necessary to support this hypothesis.



DIMER EXPLANATION 1



Fraction 1 - 5
(6 7)

Fraction 8,9

DIMER EXPLANATION 2

FIGURE 12.

HYPOTHETICAL DIMER FORMATION MECHANISM.

SECTION VIII

STRUCTURAL STUDIES OF ACETYL LIGNIN SULFONATE METHYL ESTERS (ALSME) DERIVED FROM MILLED WOOD LIGNIN

Introduction

The separation of lignin sulfonates from spent liquors of sulfite pulping processes has been studied extensively in this laboratory during previous years (3, 4, 17). The major obstacle has been repeatedly shown to be a complete separation of aromatic substances derived from sulfonation and dissolution of lignin in wood, from the sugars which are formed at the same time by acid hydrolysis of carbohydrates (17). Some sort of separation based on the ionic strength of the compounds to be separated (ion exclusion separation) seems to provide the best method so far (3, 17, 18); however, neither the lignin nor the carbohydrate part can be obtained absolutely pure and free of contaminations by the ion exclusion method. Ten-20% contaminations of the respective counterpart seems to be a reasonable goal to be achieved by ion exclusion chromatography. Complete separation of aromatic- and sugar-components and complete recovery of both parts from spent sulfite liquor (SSL), both of which are requirements for a scientific investigation of structural phenomena, had been out of reach with the methods available so far.

To get a better founded and more complete approach to our research objectives, it appeared necessary to study the sulfonation reaction (as used in the acid sulfite pulping process of wood) with a variety of models for lignin in wood (See Section VI).

We started this approach with the preparation of various LOMOLS-like monomers and dimers. Their sulfonation and transformation to acetylated

lignin sulfonate methyl ester (ALSME) derivatives taught us first the principal sulfonation pathway of compounds with different functional groups and active sites, and second, the potential use of highpowered analytical tools such as NMR- and mass-spectroscopy.

The application of these synthetic and analytical approaches to various LOMOLS-fractions secured from SSL by Sephadex G-25 separations has been discussed in Section VII in detail.

Milled wood lignin (MWL) (19-21) is the genuine plant material, isolated from wood by a special mild procedure (ball milling and dioxane extraction). It is free of carbohydrates and extractives, yet widely considered to be unchanged lignin. It is a "model" by definition, since it comprises only about 25-50% of the total lignin-part in wood, and since it has another physical form and environment than in wood. The lack of incrustation in a cell wall or between cell walls (middle lamella) affects the melting and softening behavior of the material and lets questions of accessibility appear irrelevant. The different solubility of the isolated lignin allows most reactions to proceed as homogeneous reactions (22).

The advantage of working with MWL as a model for lignin (the DP of MWL is reported to be around 60, molecular weight about 11,000 is based on the fact that MWL is a homogeneous material, free of carbohydrates and extractives. An empirical formula, based on the assumption of C_9- monomeric units, can be designed for the material and a balance of certain functional groups can be established. That means that the starting material of the reaction (e.g., sulfonation) can be well defined. Changes that occur during the reaction can be well detected and controlled.

In sulfonation reaction in particular, the sulfonates do not have to be separated from any carbohydrates. Control over all the organic material is guaranteed.

Experimental

Milled wood lignin, isolated from Western Hemlock, was sulfonated under mild conditions (100°C, pH 1.5, 24 h). The reaction product was freed from excess SO_2 and sulfate ions. The calcium salts of the lignin sulfonates were fractionated over Sephadex G-25 in water. Twelve fractions were collected and separately transformed to ALSMES.

These ALSMES were purified and investigated by NMR spectroscopy and elemental analysis. The empirical formulae were calculated for each of the 12 fractions.

Results and Discussion

Representativeness of Milled Wood Lignin: The elution diagram of Ca-LS from sulfonated MWL is compared with that of Ca-LS from a spent sulfite liquor in Figure 13.

Differences between both elution diagrams can be visualized in the very high- and the very low molecular weight region. Both differences signify the peculiarity of the MWL preparation. The highest molecular weight portion is absent since it cannot be extracted with neutral solvents from the fiber. The lowest molecular weight material and the extractives are missing as well, due to their removal prior to the isolation of the wood lignin.

Structural Phenomena

By Elemental Composition: The empirical formulae for the C_9-basis were calculated for all of the twelve fractions. They are compiled in

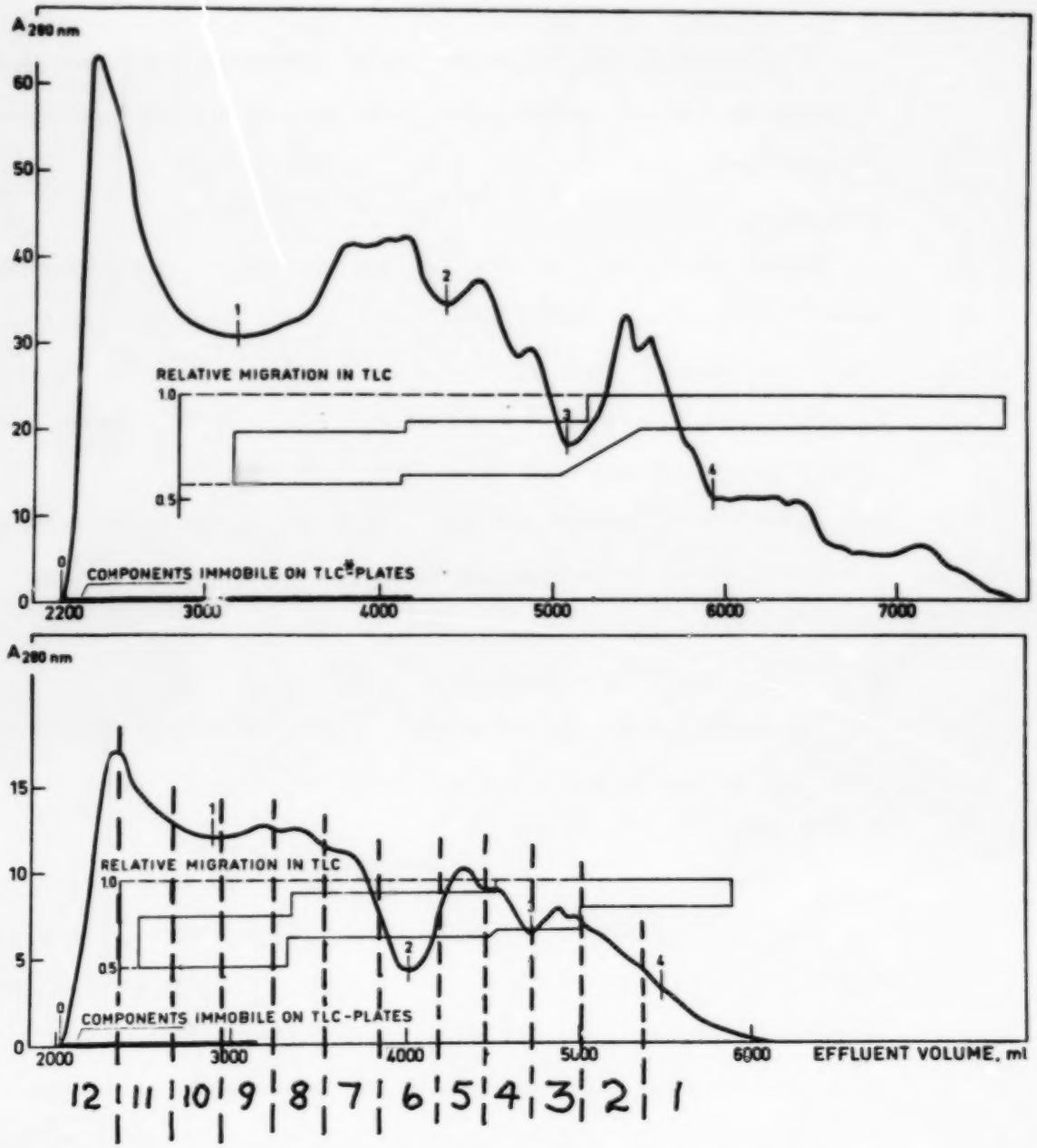


FIGURE 13.

ELUTION DIAGRAMS OF CALCIUM LIGNIN SULFONATES

Above: Secured from Spent Sulfite Liquor

Below: Prepared from Milled Wood Lignin

*TLC - Thin Layer Chromatography

TABLE 3

EMPIRICAL FORMULAE OF ALSME DERIVATIVES OF MWL

Fraction Number	C	H	O*	OCH_3	SO_2OCH_3	CH_3-CO phenolic	CH_3-CO aliphat.	Mol. W. Ave.for C_9	Σ H	Oxyg. Bal.
Original Sample	9.00	8.40	2.98	0.94	--	--	--	193.4	11.2	3.919
1	9.00	7.80	3.09	0.45	0.35	0.81	0.75	279.5	14.9	3.889
2	9.00	7.28	2.73	0.58	0.39	0.63	0.73	272.9	14.3	3.696
3	9.00	8.59	3.15	0.15**	0.40	0.50	0.80	265.9	14.1	3.703
4	9.00	7.09	2.77	0.65	0.46	0.49	0.80	279.5	14.3	3.889
5	9.00	7.23	2.77	0.68	0.46	0.42	0.70	272.5	14.0	3.906
6	9.00	7.12	2.60	0.67	0.51	0.45	0.78	279.2	14.3	3.779
7	9.00	7.44	2.73	0.73	0.49	0.44	0.81	282.4	14.9	3.950
8	9.00	7.22	2.71	0.66	0.48	0.41	0.82	278.0	14.3	3.854
9	9.00	7.26	2.56	0.76	0.48	0.37	0.76	274.2	14.4	3.798
10	9.00	7.57	2.61	0.69	0.45	0.33	0.80	270.9	14.4	3.750
11	9.00	7.50	2.73	0.71	0.36	0.29	0.60	254.3	13.4	3.802
12	9.00	7.26	2.89	0.70	0.33	0.34	0.81	264.4	13.8	3.920

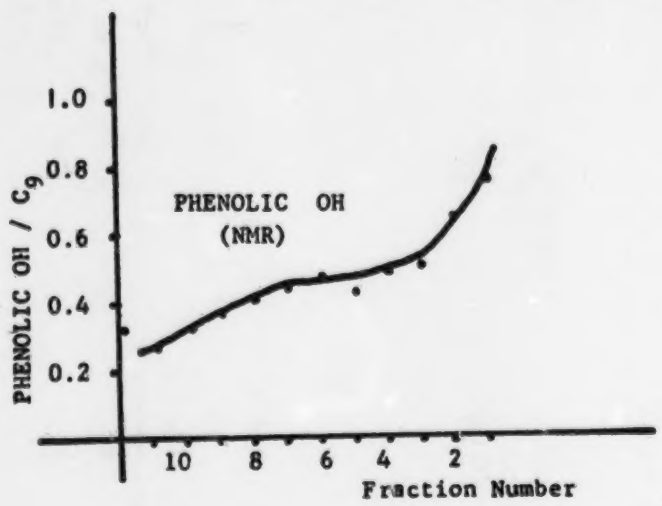
* Oxygen-determination by difference.

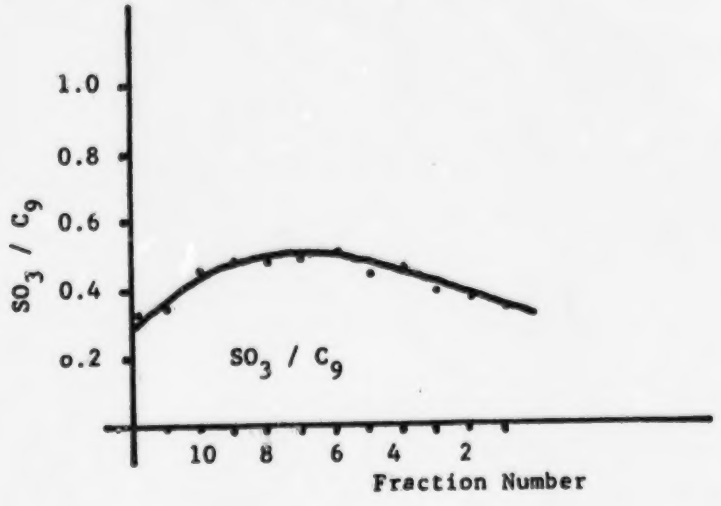
** After repeating the analysis for F_3, the OCH_3-content is about 0.60 per C_9.

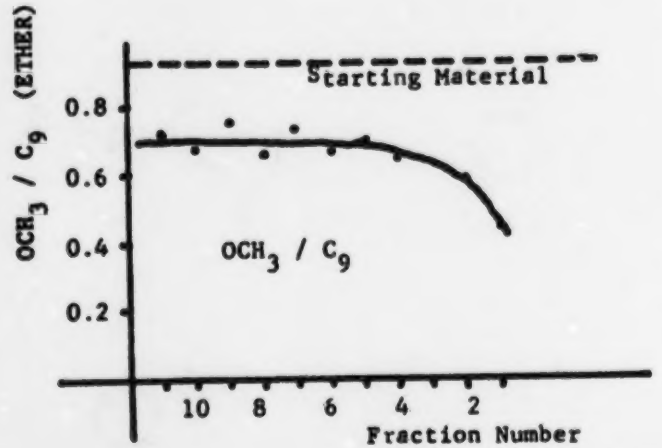
Table 3. The phenolic- and aliphatic- acetyl groups were determined by NMR spectroscopy. The most essential functional groups were plotted in diagrams (Figure 14). It is demonstrated that the content of phenolic hydroxyl groups increases from high to low mol. w. material. The increase seems to be steady in the high molecular weight range, exponential in the low molecular weight range. The content of phenolic OH groups in MWL has been reported to be about 0.3 per C_9 unit. Apparently phenolic hydroxyls are generated by the splitting of mainly α-arylether bonds (Figure 15); however, the sharp increase of phenolic OH-groups in the LMWLS region (fraction 6-1) cannot be explained only by this type of ether splitting, but must involve some other reactions.

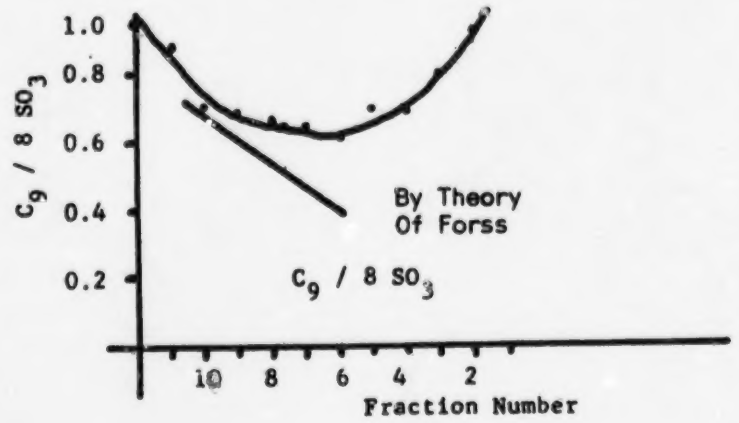
The diagram of ether-OCH_3-groups per C_9 unit signifies this other mode of phenolic OH group formation. The content of methoxyl groups remains constant in the high molecular weight LS region, but drops off drastically in the LMWLS region. This means that methoxyl groups are cleaved by the sulfonation reaction under the formation of phenolic hydroxyl groups in 3-position (23). Sulfonate groups have been reported to increase steadily for high molecular weight LS (24). This could not be verified. In contrast, the S-content of the various fractions as well as the content of SO_3-groups per C_9 unit exhibits a bell-shaped curve with the culmination point in fraction 6. Forss derived a lignin formulation from the fact that the S-content for high molecular weight lignin sulfonates is steadily increasing (25). He calculated that units of 10-18 C_9 units share each eight SO_3 groups. A similar calculation resulted in 15-20 C_9 units for every eight sulfonate groups in the same region of the elution diagram of MWL.

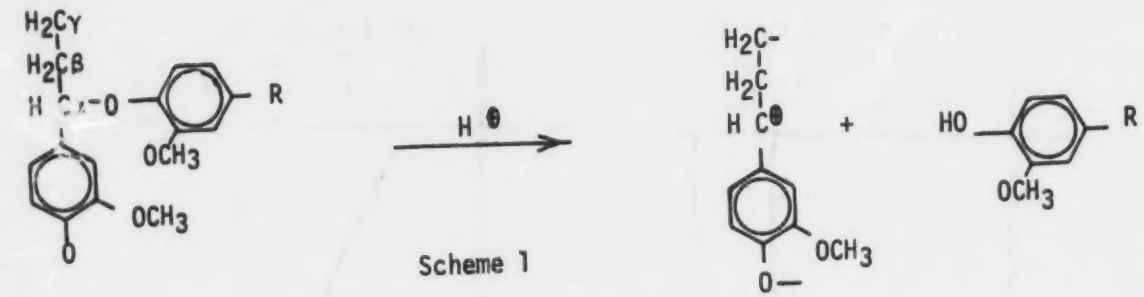
FIGURE 14. ELEMENTAL AND FUNCTIONAL GROUP COMPOSITION OF MWL - ALSME.



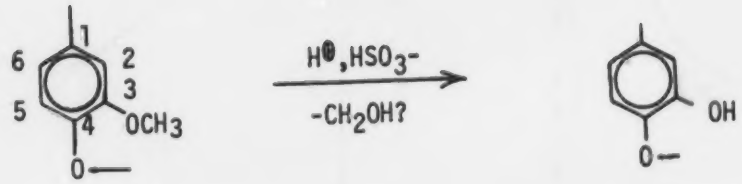




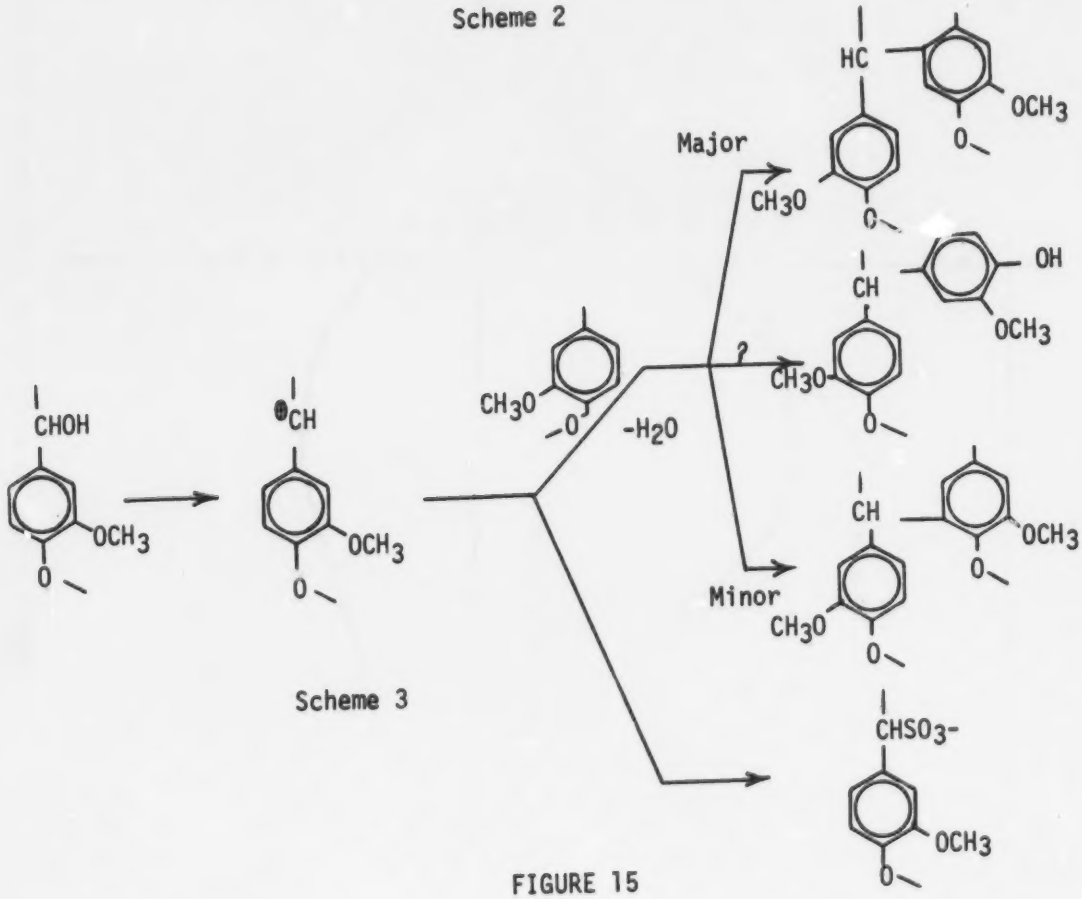




Scheme 1



Scheme 2



Scheme 3

FIGURE 15

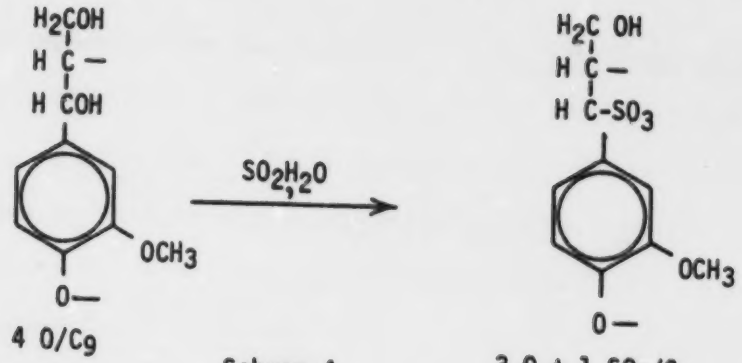
By Oxygen Function: It is well known that condensation reactions compete with sulfonation reactions (Scheme 3, Figure 15) (26). In the sulfonation reaction, a hydroxyl group is substituted by a sulfonate group whereas condensation means elimination of a molecule of water.

A balance of oxygen atoms per C_9 unit for all fractions is able to give hints for the location of condensed LS in the Sephadex diagram. For this reason all oxygen atoms per C_9 unit are added up (Scheme 4, Figure 16). If a sulfonate group has substituted an OH-group, it is counted as 1 oxygen. The oxygens attached to the sulfur atom are not counted.

The MWL starting material had 3.92 O/C_9 (see Table 3). The deviation from this value for each of the 12 fractions has been calculated and plotted in Figure 17. All oxygen values lower than 3.92 O/C_9 indicate condensation (or other loss of water).

The shape of the curve in Figure 17 indicates that condensed LS are "located" in the high- as well as the low molecular weight material, whereas the material right between both areas (fraction 6) indicates no condensation at all. Interestingly, this fraction is also the fraction with the highest SO_3 content of all. Condensed LS are found mainly in fractions 2 and 3 and 9-11. The degree of deviation of oxygens from the starting material (0 - 0.25 O/C_9) can be considered to be an expression of the degree of condensation. Thus, fractions 2 and 3 contain LS, where every 4. or 5. aromatic ring is condensed onto the side chain of another unit (probably mainly in 6-position). Both fractions comprise about 10% of the total lignin sulfonates.

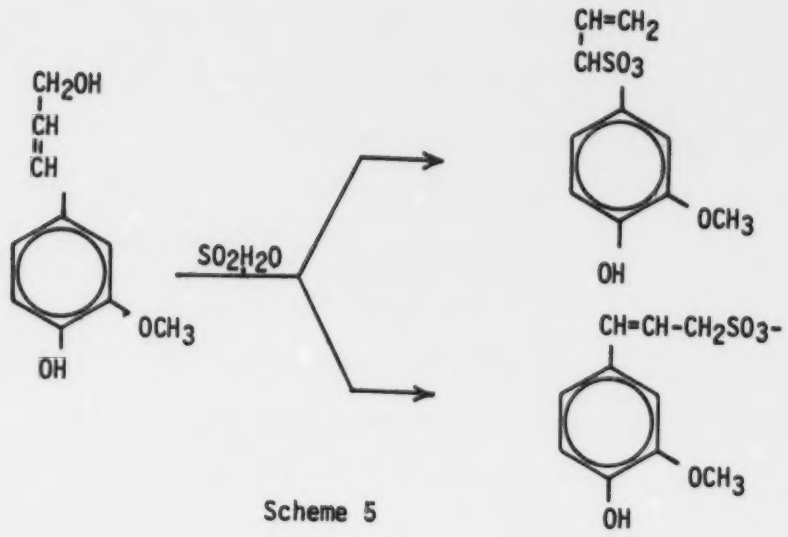
By NMR-Spectroscopy: This method has been shown to be a powerful instrument in the analysis of lignin model compounds as well as lignins



4 O/C_9

Scheme 4

3 O + 1.SO_3/C_9



Scheme 5

Scheme 6

FIGURE 16

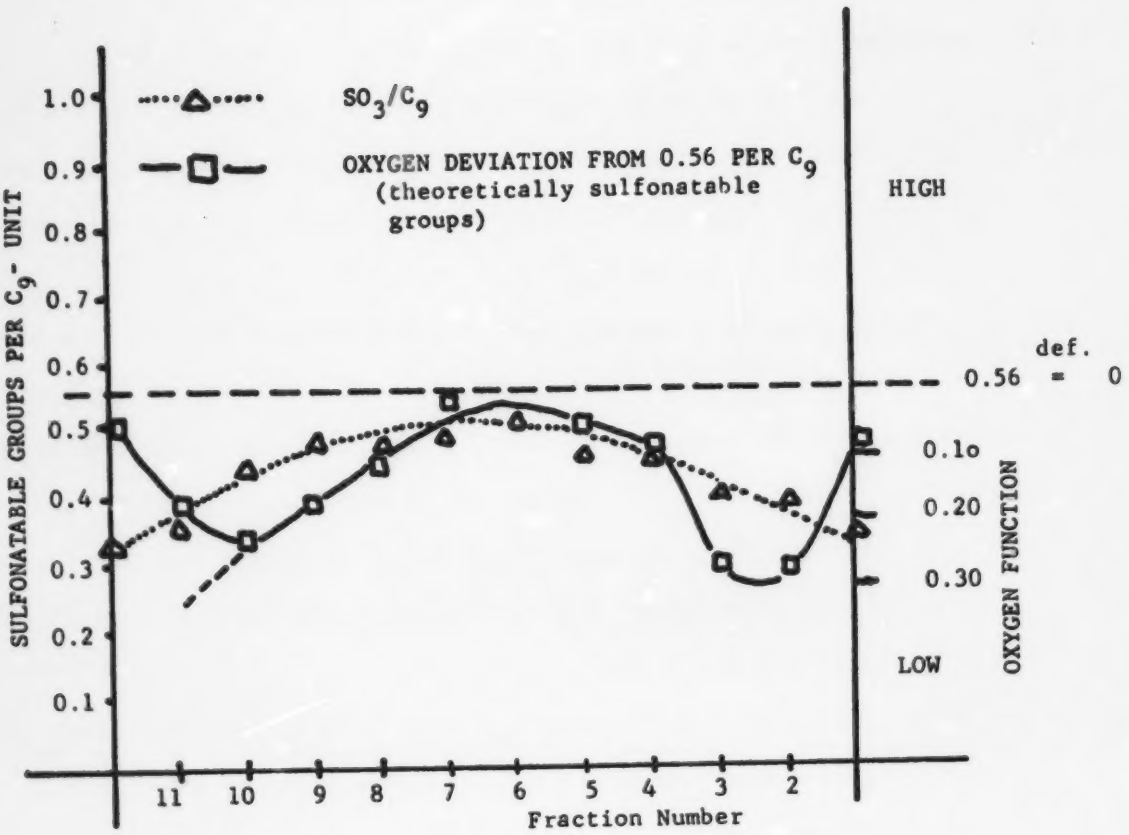


FIGURE 17

OXYGEN FUNCTION IN ALSME DERIVATIVES CF MWL.

(see Section VI and VII). Side chain configurations as well as ring substituents can be estimated or determined with high accuracy.

The application of NMR spectroscopy to the 12 fractions of the MWL fractionation revealed some more specific features of the structure of LS. Fractions 2 and 3 proved to contain some sulfonation products of coniferyl-alcohol (Scheme 5, Figure 16).

These compounds, however, were very probably not present as monomers but rather attached to oligomeric fragments as indicated by thin layer and Sephadex LH-20 chromatography. The C-6 atom of the aromatic ring is the position of intermolecular linkage. Since both vinyl-compounds have only three oxygen functions per C_9, not all oxygen deviation shown in Figure 17 can be due to condensation; some of it must be due to these unique side chain configurations.

The NMR spectra of fractions 8 upward show signs of increasing intensity of carbon-carbon condensation in position 5 of the aromatic ring. Condensation has been indicated also by the oxygen deviation of these fractions, shown in Figure 17.

Conclusions

Sephadex chromatography, elemental analysis and NMR spectroscopy helped to elucidate some of the structure of LS.

It was shown that phenolic hydroxyl-groups are formed through demethylation of aromatic methoxyl-groups (Scheme 2, Figure 15). In the lowest molecular fraction of the lignin sulfonates from MWL, about every second C_9 unit was demethylated. The para positions of these newly formed phenolic hydroxylgroups (position 6 of the C_9 unit) are the most activated nucleophilic sites of the entire lignin (Scheme 6, Figure 16).

These nucleophiles are, therefore, the most probable ones to undergo condensation with carbonium ions (Scheme 3, Figure 15), thus contributing to the lowering of the methoxyl content of the lignin sulfonates. The sulfur content of LS is represented by a bell-shaped curve, with the culmination point at the border between high- and low molecular weight LS. In LMWLS the sulfonation products of coniferyl alcohol have been found, probably attached to oligomeric fragments of lignin through the 6-position of the aromatic ring.

Summary

Milled wood lignin (MWL) of Western Hemlock has been mildly sulfonated and analyzed by Sephadex G-25 chromatography, NMR spectroscopy and elemental analysis. A comparison of SSL from pulping of wood chips was found to be in good agreement with the sulfonated MWL in all essential parts, except for the very low- and very high molecular weight range. Results from the MWL are, therefore, believed to be representative also for the lignin from wood.

An exact division between low- and high molecular weight LS is proposed by employing Sephadex chromatography, solubility in ether and elemental analysis. According to this division, MWL is composed of 30% LMWLS and 70% high molecular weight particles.

Structural studies revealed the content of phenolic hydroxyl-, methoxyl- and sulfonate-groups. The degree of condensation was estimated. Certain unique structural configurations could be assigned to some of the LMWLS. An explanation for the demethylation observed was proposed,

SECTION IX

STRUCTURE AND REACTIVITY OF LOMOLS

Introduction

The structure of LS has been elucidated so far nearly exclusively on the basis of either chemical reactions of lignin models, or physicochemical characterizations of LS by gel permeation chromatography (see Section V, VI). It has been our objective in this research enterprise to separate these isolated LS into a series of fractions each of which consist mainly of particular mers, such as monomers, dimers, trimers, and then mixtures of higher mers, and to characterize each mer class by several methods (see Section IV).

We approached these objectives by purifying and fractionating LS from a SSL over Sephadex G-25 (see Section V). Characterizations by UV and IR preceded the transformation of the various LS fractions to ALSME derivatives (Section VII). The analytical basis for the characterization of such ALSME derivatives had been created using LOMOLS-like monomers and dimers as models (Section VI). The use of Milled Wood Lignin as an "advanced model" for wood allowed us to make concise statements about the elemental composition of lignin before and after sulfonation, and the fate of most functional groups during this reaction (Section VIII).

From the literature and from our earlier studies, we knew that LOMOLS appeared different from the rest of the LS by a number of characteristics, as reflected in various chemical and physicochemical methods. The most important ones of them shall be listed in the following.

Forss and coworkers found that the UV absorption of the LOMOLS suffered a severe red-shift when the solution was alkalized (7). The UV spectrum of HIMOLS appeared unaffected by the pH. This Finnish group also reported findings which demonstrated a significant difference in solubility in sodium bisulfite solution of pH 5.5 - 6 between LO and HiMOLS (6). Fractionation of LS over ion exchange resin (DOWEX 50W-X2) separates the LOMOLS from the HIMOLS (see Section V). This separation can be visualized also by the color: LOMOLS are yellow, whereas HIMOLS are brown. Fractionation on Sephadex G-25 yields an elution diagram similar to that from Dowex. Very significant differences bewteen low and high molecular weight parts of LS can be observed in the elemental and functional group composition, as discussed in earlier sections of this report (Section V, VII, and VIII).

As to the origin of these differences that are in apparent disagreement with the conventional understanding of lignin, (particularly concerning its homogeneity) (see ref. 27-29), Forss and coworkers believe that lignin is as heterogeneous as the carbohydrate portion in wood (cf. hemi- and holo-cellulose). Other theories explain these differences with secondary condensation of lignin material evoked by acid hydrolysis.

To find a better understanding of the structure, origin, and reactivity of LOMOLS we undertook two-fold studies:

a. We isolated lignin sulfonates into a series of fractions each of which consisted of particular mers, and we characterized each mer class by several methods; and

b. We sulfonated the hydrolysis products of the most representative dimeric lignin model compound and investigated the reaction products.

Experimental Section

Lignin sulfonates from a spent sulfite liquor were purified as usual (cf. Section V) and fractionated by ion exclusion chromatography. The LOMOLS comprising part was neutralized, concentrated and freeze dried. The total portion was transformed to ALSME derivatives in accordance with the procedure described in Section VI.

About half of the ALSME were fractionated over Sephadex LH-20 using methanol as mobile phase. The elution diagram exhibited a number of well separated individual peaks, the corresponding material of which were collected and recovered by evaporation and drying under vacuum. The separation had a very high degree of reproducibility. The substances from each individual peak area were refractionated under the same conditions as used before (see Figure 18). Whereas some peaks proved the high separation potential of this method (peak d) other peaks exhibited elution diagrams very similar to the total ALSME fraction (peak c). Material from corresponding mer classes (peak areas) was re-collected and recovered by evaporation and drying. The oily residues were dissolved in a little methanol and precipitated from ether 10:1. The ether solubles (ES) and the ether insolubles (EIS) were recovered separately. The samples were analyzed by NMR spectroscopy and elemental composition.

The other half of the ALSME derivatives was dissolved in chloroform, and brominated under very mild conditions (temp. below 5-10^{o}C) until there was no more discoloration of the bromine. Then the material was recovered and treated the same way as outlined above for unbrominated ALSME.

Veratryl-glyceryl-β-arylether was submitted to a mild acid hydrolysis (ACIDOLYSIS) following the procedure of Adler et. al. (30, 31) (0.2 N HCl in dioxane:water 9:1; reflux for four hours). At that time, no more unreacted

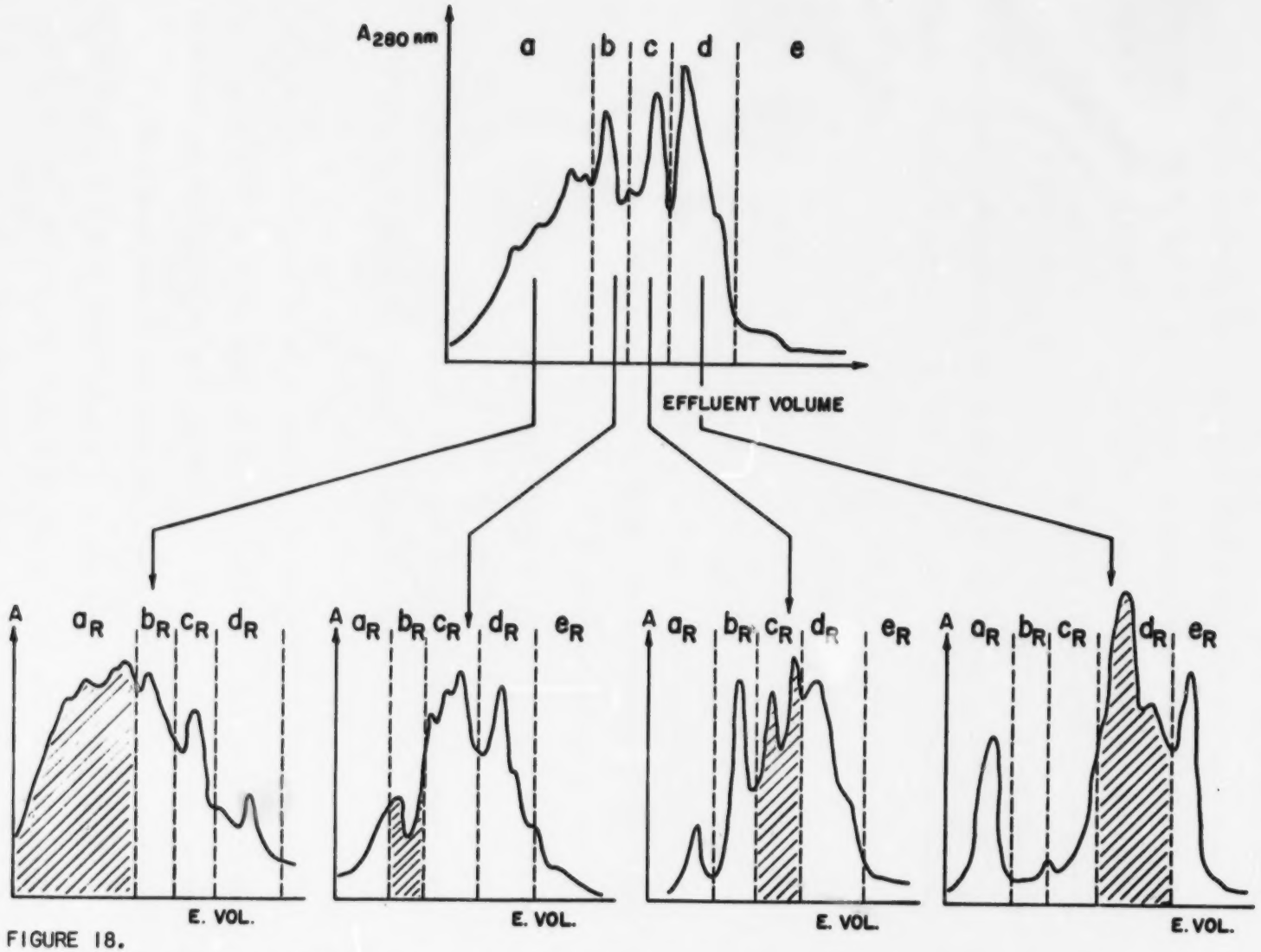


FIGURE 18.

ELUTION DIAGRAM OF ES ALSME DERIVATIVES AND THE REFRACTIONATION PATTERS OF SOME MER CLASSES.

β-ether was left, as indicated by TLC. Without cooling, excess sodium bisulfite in little water was added to the solution and reflux continued for two days. Afterwards the solution was concentrated under vacuum and redissolved in water. The aqueous solution was extracted several times with plenty of chloroform to remove the chloroform solubles (unsulfonated organics). About 2/3 of the starting material was recovered in the organic phase. The rest (aqueous solution) was concentrated and run over an ion exchange column in H-form to liberate the anions. The free acids were freed from SO_2, neutralized, concentrated and freeze dried to yield mainly the sodium salts of the sulfonated hydrolysis products of the β-ether. The usual transformation (cf. Section VI) yielded the ALSME derivative which was divided into an ether soluble and an ether insoluble part by precipitation from ether. All steps were controlled by TLC, Sephadex G-25 and LH-20 chromatography, IR- and NMR-spectroscopy, as far as possible. The elemental composition of the EIS ALSME derivative was determined and its empirical formula calculated.

Results and Discussion

The refractionations were undertaken with the idea in mind to prove the potential of isolating individual compounds from LH-20 separations. This potential seemed to be very high, since the peaks exhibited were tall and slender, indicating excellent separations (see Figure 18, 20). Also NMR spectroscopy indicated good separation of the individual fractions.

However, upon refractionation most mers or mer classes did not exhibit elution diagrams with single peaks, but demonstrated a rather peculiar instability, indicated in the "regeneration" of certain other peaks; as if there was an equilibrium of the compounds represented by these peaks. By

naming all peaks according to their retention volume, certain "regeneration pathways" could be established (see Figure 19).

The isolation of compound c seemed to be most likely for several reasons: The NMR-spectrum indicated the predominant presence of a compound with a typical vinyl side chain. This compound had earlier been found to comprise the major portion of the LOMOLS. TLC demonstrated that this compound was present as monomer (comparison with an authentic sample with this structure). However, the refractionation of peak c yielded the desired material only in minor amounts, and the fractionation pattern exhibited resembles strikingly much the original elution pattern of the ES ALSME derivatives.

These results allowed the conclusion that some compound, possibly the one with the β-vinyl structure, rearranges and/or isomerizes extremely readily to nearly everything we call LOMOLS. Since the isomerization behavior of compound I had been studied before, a reaction pathway involving a double bond was suspected; therefore, the second half of the ES ALSME was brominated prior to its fractionation.

After bromination, the only obviously altered peak appears to be d. It is interesting to note that this peak was found to be the direct precursor of the polymerization (vinyl-plym.), as stated from the previous experiment (see Figure 19).

Refractionation of the mer class representing compound d, resulted indeed in the isolation of a single, stabilized peak d. The refractionation of the other mer classes showed again the known instability (see Figure 21). No satisfactory explanation was found for the observation that the vinyl compound I was again present in the brominated ES ALSME. There is no reason

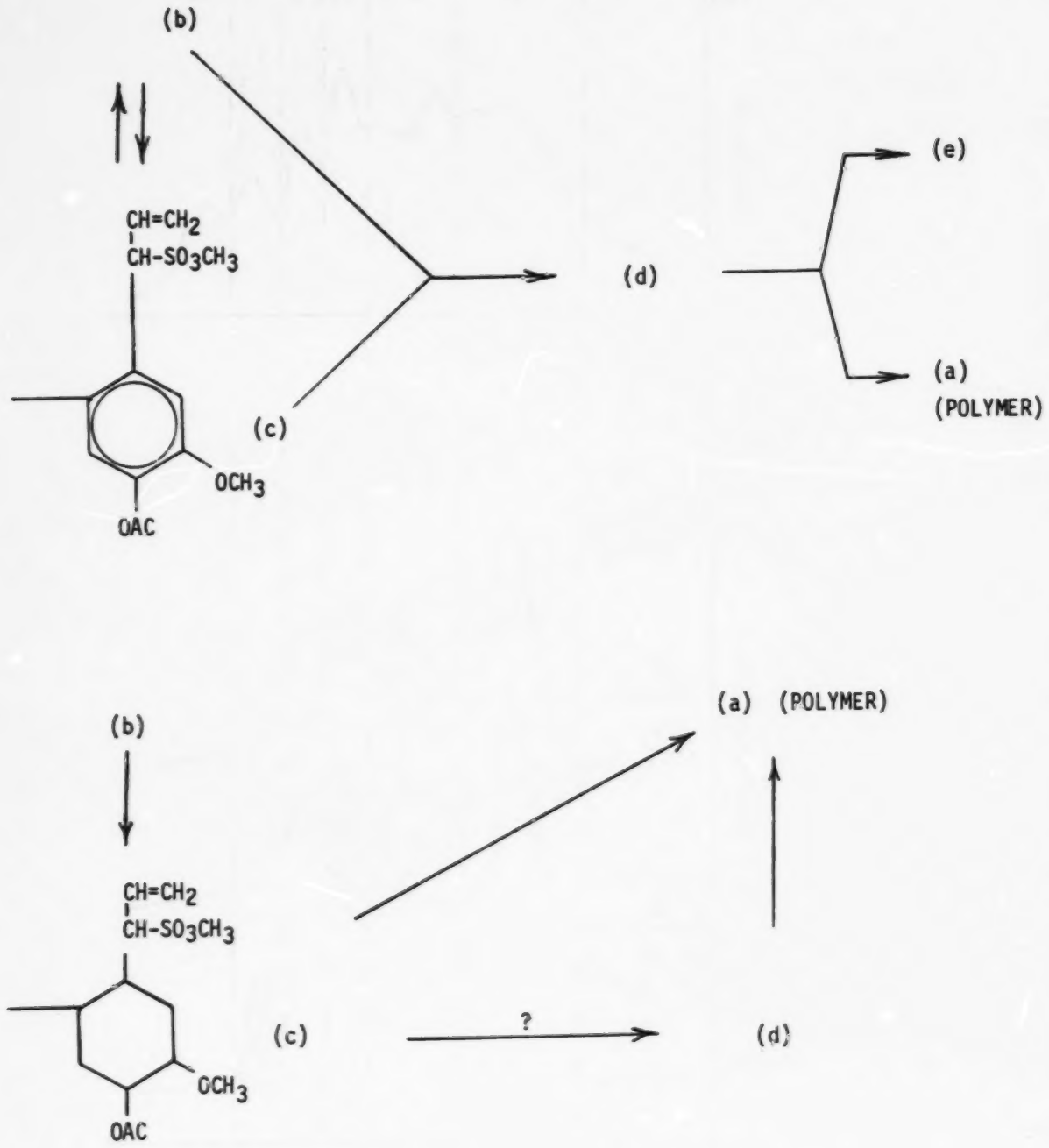


FIGURE 19.

THE "REGENERATION PATHWAY" IN TERMS OF PEAKS, AS VISUALIZED FROM THE SEPHADEX LH-20 FRACTIONATIONS.

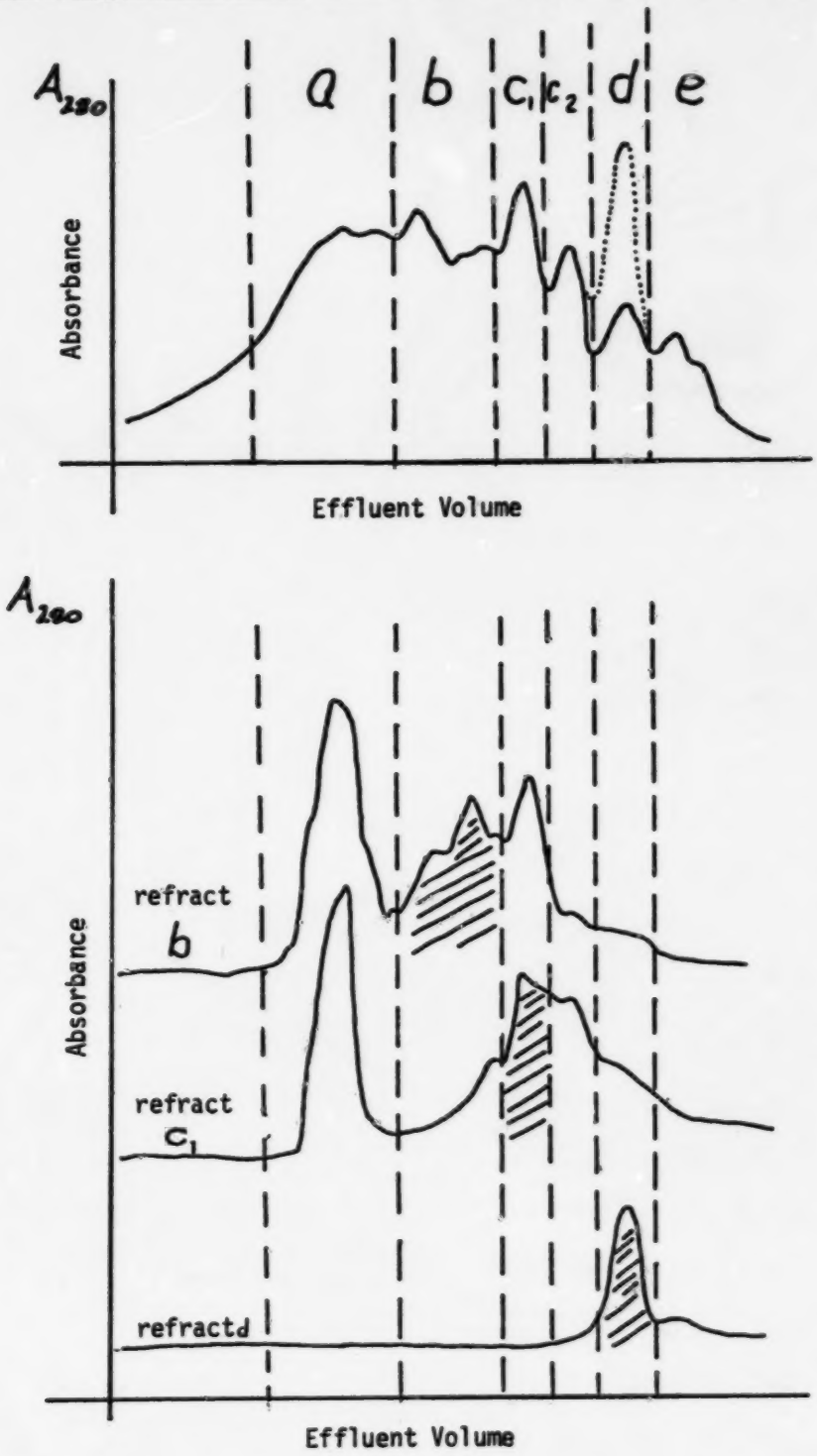


FIGURE 20.

Elution Diagram of Brominated ES ALSME Derivatives and the Refractionation Patterns of Some Mer Classes.

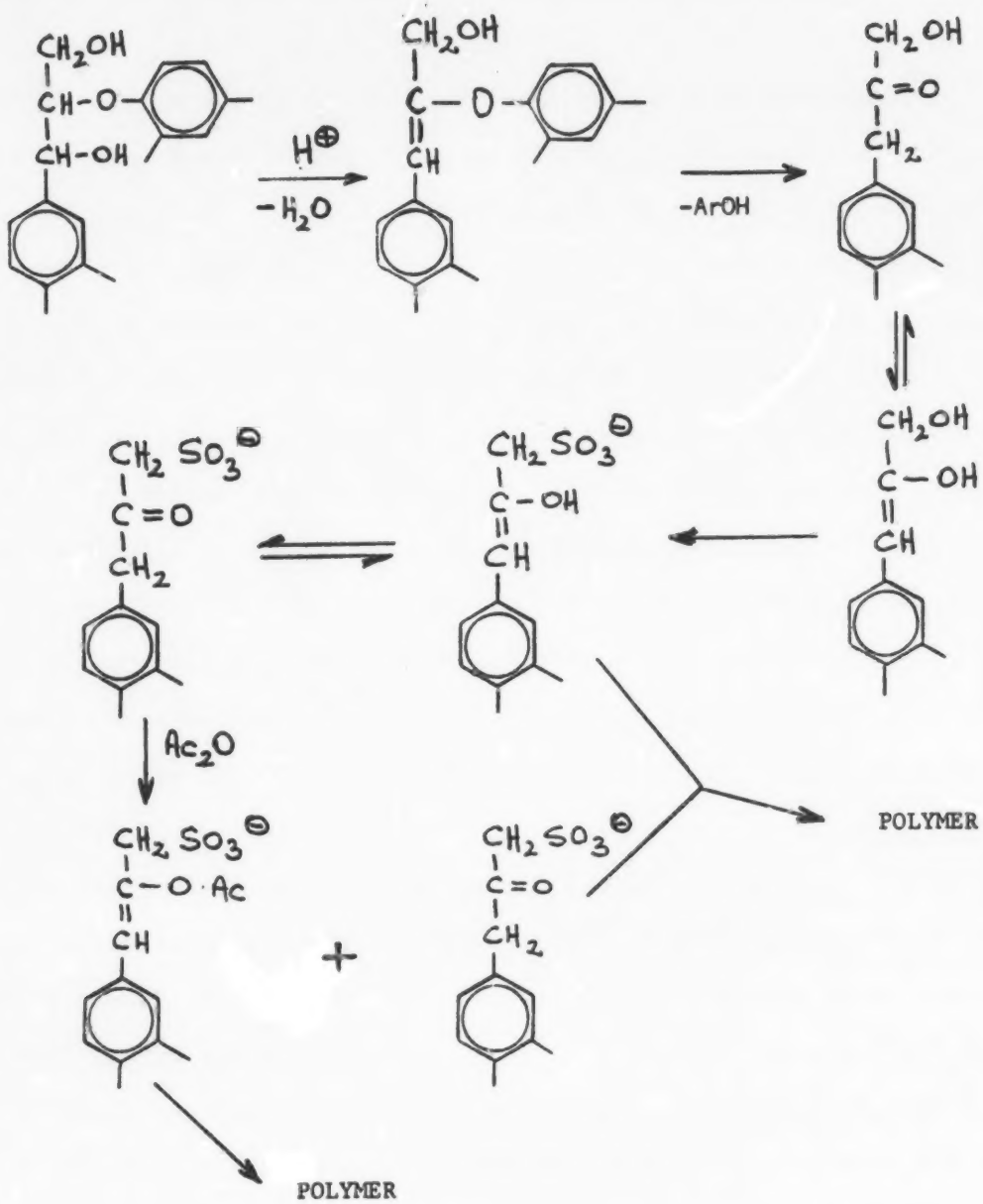


FIGURE 21.

ACID HYDROLYSIS OF THE VERATRYL-GLYCERYL-β-ARYLETHER, ITS SULFONATION- AND POLYMERIZATION PRODUCTS.

why its double bond should survive bromination. It seems as if this double bond is generated not only by coniferyl alcohol endgroups as was assumed hitherto.

The question as to the origin of the vinyl-groups is believed to be answered only unsatisfactorily with coniferyl alcohol end-groups. It could be demonstrated that the most abundant intermediate of the acidolysis reaction pathway of the β-ether, as described by Adler, et.al., was able to form a sulfonate with bisulfite ions (see Figure 21). This compound formed two spots on TLC after acetylation with acetic anhydride and pyridine. Since it also contained a carbonyl-group, its structure is expected to match compound II and acetylation forms the enolacetate III and the keton II.

After transformation to ALSME-derivatives, 85% of the sulfonate appeared as polymeric ether insoluble amorphous powder. The residual 15% ether-solubles, showing eight distinct spots on TLC, exhibit an abnormal amount of vinyl-groups in their NMR-spectrum (most of them conjugated to the aromatic ring) and an acetyl-peak, the location of which matches an enolacetate.

Conclusions

LOMOLS seem to be a mixture of several particular mers and mer classes that are in an equilibrium with each other and that rearrange and/or isomerize easily to "regenerate" this mixture. The main reaction apparently involves a double bond, which indicates vinyl polymerization as the mode of formation of higher mers. Guaiacyl-propene-2-sulfonic-acid-I seems to play a key role in this reaction. Its double bond stems obviously not only from coniferyl alcohol endgroups.

It was found that hydrolysis products of β-ethers undergo sulfonation, and that these sulfonates are capable of forming vinyl groups via keto-enol tautomerism, where the enol form polymerizes readily, probably in a vinyl-type polymerization.

SECTION X

PRELIMINARY STUDIES ON INDUSTRIAL SCALE SEPARATION OF SSL COMPONENTS

Introduction

In the light of the investigations reported in the earlier sections of this report and prior work in this laboratory, preliminary experiments were carried out directed toward investigation of feasibility of industrial scale separation of SSL components.

Two techniques were given preliminary study. One was solvent extraction of spent sulfite liquor solids by butanol-water and ethanol-water mixtures. The other technique was a further study of the use of ion-exchange resins on a column arrangement. LOMOLS are separated from HIMOLS presumably through some molecular sieve mechanism and from sugars in recognition of the fact that the non-ionic sugars would not be excluded from the pores in an ion-exchange resin, whereas the lignin sulfonates would be so excluded.

Earlier work in this laboratory showed that high molecular weights lignins can be separated from the rest of the substances in SSL, perhaps technically although not necessarily economically by dialysis (31) and by the formation of amine salts of lignin sulfonates. Quantitative separation from sugars was achieved by precipitation or solvent extraction of the amine salts of lignin sulfonates. This salt formation was reversed by use of sodium hydroxide or another strong base to give rise to freeing the amine and formation of the metal salts of the lignin sulfonates which is perfectly soluble in water.

Separation by Alcohol Extraction

In this laboratory, a considerable effort has been made toward separating lignin sulfonates of various molecular weight (32-36). One of the approaches has been the fractionation by ethanol-water mixtures (37). It could be demonstrated that the MW of the dissolved fractions increased with the water content of the eluant. Preliminary tests have shown that SSL solids can only be extracted with ethanol-water mixtures with water contents up to 15%. At higher water contents, the solids yielded a sticky unextractable tar.

Experimental

Steam-stripped fermented spent sulfite liquor from gymnosperms was provided by the Georgia-Pacific Corp., in Bellingham, Washington. The liquor was neutralized with sodium hydroxide, freeze dried, and then extracted with ethanol-water mixtures beginning with 95% ethanol and followed by exhaustive extraction with ethanol containing increasing proportions of water. The extracts and extracted solids were analyzed by Sephadex gel chromatography applying controlled ionic strength and the results are shown in Table 4.

In a second similar experiment spent sulfite liquor solids (Calcium base) were extracted with ethanol and ethanol-water mixtures and the weight of the dissolved solids and the absorbance of solutions of the same was determined. A material balance by weight and absorption at 280 nm was established.

Results and Discussion

Treatment with 95% ethanol resulted in the extraction of about 2% of the organic matter which contained only 25% UV absorbing material (see

TABLE 4

Extraction Conditions: 10g CaSSL extracted with 500 ml. ethanol and ethanol:water mixtures for twelve hours at room temperature.

	a (g)	b %	c Aa	d $\%A_{280}$	e (g)	f %s	g %r
Starting Material	10.0	100.0	9.100	100.0	7.6	76.	76.
Ex 100	0.6	6.0	0.022	0.24	0.018	0.12	3.0
Ex 95	0.9	9.0	0.184	2.0	0.15	1.5	16.7
Ex 90	1.50	15.0	0.980	10.8	0.82	8.2	54.5
Ex 85	2.20	22.0	1.430	15.8	1.19	11.9	54.5
Ex 80	3.20*	32.0*	1.430	15.8	1.19	11.9	37.2*
* Tarry residue							
Precipitation (dissolved matter)							
Starting Material	20.00	100.0	18.2	100.0	15.20	76.0	76.
95-S	4.48	22.5	2.40	13.3	2.02	10.2	45.
90-S	6.00	30.0	3.65	20.0	3.05	15.2	50.
80-S	8.20	41.0	6.40	35.0	5.30	26.5	64.

Column Explanation

a weight (g) recovered.
b % weight based on starting material.
c average absorbance of samples.
d % of total absorbance at 280 nm.
e theoretical weight of absorbing material where a_{280} assumed constant at 12.0.
f % weight of absorbing material (LS) based on starting material. 1/b X(e X 100)
g % weight of absorbing material (LS) based on recovered fraction.

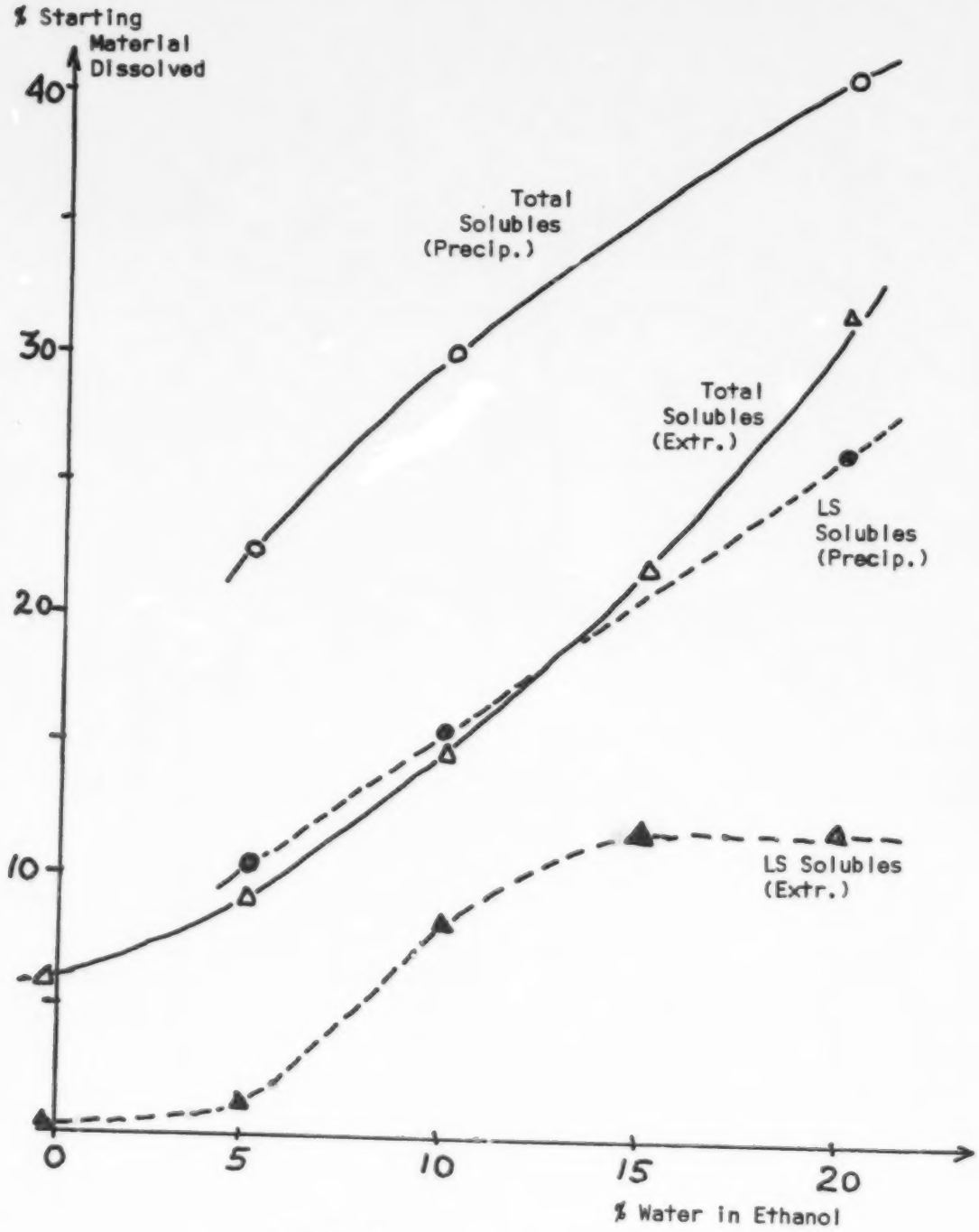


FIGURE 22.

DISSOLUTION OF SSL SOLIDS AS A FUNCTION OF THE WATER CONTENT OF ETHANOL.

Figure 22). Two-thirds of the material represented carbohydrate degradation products. The extracted lignin sulfonate fraction gave a well resolved elution pattern on gel chromatographic separation using Sephadex G-15. It could be demonstrated that the so-extracted LOMOLS fraction consists mainly of very low molecular weight material.

Subsequent extraction with 88% ethanol, carried out in three stages, resulted in the dissolution of 22% of the organic matter consisting of about 50% of the lignin sulfonates. The ratio between absorbing (lignin-like) and non-absorbing (carbohydrate-like) material in all three stages was practically the same. The elution diagrams obtained from analytical Sephadex separations indicate that these fractions consist also of very low molecular weight materials.

In another set of experiments we studied the precipitation of lignin sulfonates from aqueous solutions by ethanol. We established the material balance and found that the ratio between carbohydrate degradation products and lignin sulfonates over the investigated range of ethanol-water mixtures (up to 20% water) remained constant. Comparative gel-chromatographic LOMOLS obtained by the two different techniques (extraction and precipitation) have a very similar composition.

Conclusions

These preliminary studies allow the conclusion that a certain amount of carbohydrate degradation products (e.g., monosaccharides) can be extracted with absolute ethanol, or generally, high percentage ethanol (beyond 95%); however, a satisfactory separation of lignin sulfonates and carbohydrate reaction products by more extraction with, or precipitation from, ethanol water mixtures does not seem to be feasible.

Separation by Ion Exclusion

Several years ago in this laboratory, pioneering research had been conducted on sugar lignin sulfonate separations using ion exchange resins (17). A fractionation of SSL components into sugar-rich and lignin sulfonate-rich portions was achieved. In recent years, this technique has been studied and improved in several academic and industrial laboratories (7, 38). To evaluate the separation under optimal conditions, we carried out some studies applying extremely low loadings and very slow flow rates and established material balances.

Experimental

Freeze dried spent sulfite liquor was dissolved in distilled water, loaded onto a column filled with Dowex 50W-X2, samples eluted with water, and fractions collected with an automatic fraction collector. These were examined using UV absorption at 280 nm and the elution diagram was plotted (see Figure 23). Each fraction was quantitatively neutralized with sodium hydroxide, thus determining the acid number. The neutralized sodium spent sulfite liquor was then concentrated, freeze dried, and the dry matter content of each sample was determined. The elution diagrams from analytical gel chromatography using Sephadex G-25 and G-50 were also determined.

Results and Discussions

By using the ion-exchange resin in H-form we expected a better separation of charged molecules, such as lignin sulfonates, aldonic acids and sugar sulfonic acids, from non-charged molecules such as mono-, di- and poly saccharides. Due to the low loading and slow flow rate, we observed complete exchange of ions within the first 5 to 10 inches of the total column length (7 feet). The remaining separation was exclusively governed by the principle of ion-exclusion.

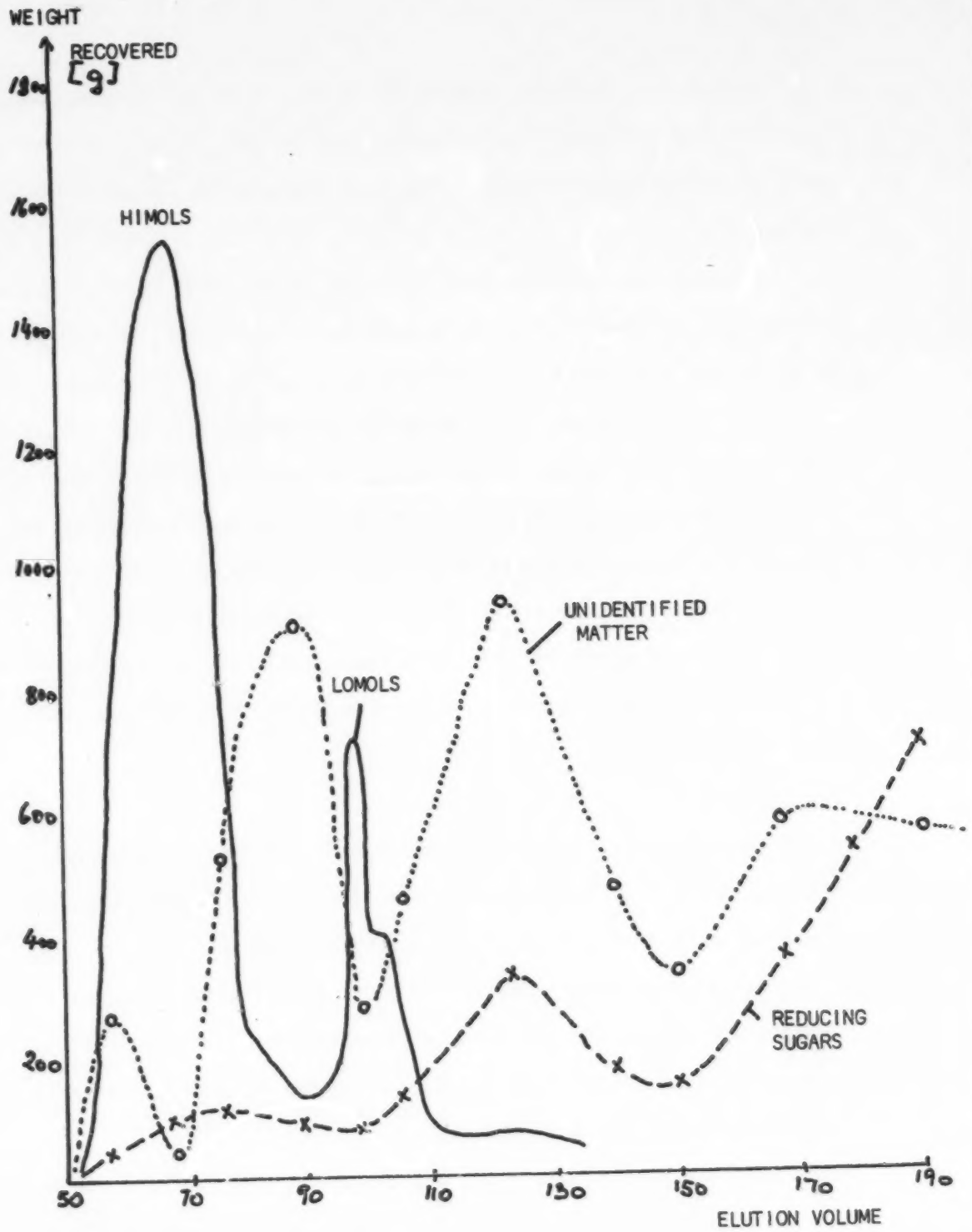


FIGURE 23.
ELUTION DIAGRAM OF A SSL IN ION EXCLUSION CHROMATOGRAPHY.

Although no complete categorical separation of the SSL components could be achieved, the accomplished separation into three main fractions with distinct different composition is of enormous technical and scientific interest.

The main fraction comprises about 55% of the total material and contains high and middle molecular weight lignin sulfonates, contaminated with carbohydrate degradation products to an extent of about 10% or less.

The second fraction consists of low and middle molecular weight lignin sulfonates and carbohydrate degradation products in about equal proportions (1:1). Since the content of reducing groups is moderate, we assume that the non UV absorbing material consists mainly of aldonic acids. The fraction amounts to about 15% of the total material.

The remaining 30% contains only traces of lignin sulfonates and comprises carbohydrate degradation products with the bulk of mono- and disaccharides.

Conclusions

The reported preliminary study demonstrated that a crude separation of spent sulfite liquor components into three major fractions by ion exchange exclusion chromatography can be achieved.

From an industrial point of view, all of the three fractions have a utilization potential. The last two fractions contain practically all of the carbohydrate degradation products which may represent a valuable source for a number of salable products.

Both experiments gave rise to preferential separation of low molecular weight lignin sulfonates. This is indicated in the graphs of Figure 22 and Figure 23. In the separation with alcohol, 10% of the lignin sulfonates

were separated and proved to be of low molecular weight and relatively low content of sugar components. In the unique ion exclusion separation similar results were obtained as shown in Figure 23.

Of special interest in most cases are the results obtained on separation of the resultant low molecular weight lignin sulfonate fraction when subjected to gel chromatography using Sephadex G-25 or Sephadex G-50.

The results from the ion exclusion experiment demonstrated that three different types of fractions could be obtained:

Fraction I represented about 55% of the total material containing high and medium low molecular weight LS contaminated with carbohydrate degradation products up to 15%.

Fraction II consisted of low to middle molecular weight LS and about 50% carbohydrate degradation products, most of which were non-reducing and of unknown structure (possibly aldonic acids).

Fraction III contained mainly sugars.

SECTION XI

CONCLUDING COMMENTS

Lignin sulfonates from the spent sulfite pulping liquor of Western Hemlock have been separated in purified form using an alcohol extraction and precipitation method as well as ion exclusion chromatography. Both methods resulted in the isolation of mainly three fractions: high and medium molecular weight lignin sulfonates, low mol. v. LS, and carbohydrate degradation products. In the alcohol extraction and/or precipitation method, the separation was of lower quality than in the separation by ion exclusion. Neither separation method, however, was capable of isolating pure LOMOLS.

The characterization of lignin sulfonates worked best after covering the highly polar hydroxyl- and sulfonate-groups by acetylation and esterification. NMR spectroscopy allowed a complete functional group analysis and overall, appeared to be the most useful analytical method.

Summarizing the structural evaluation of low and high molecular weight LS, it appears that LOMOLS are different from HIMOLS by configurations that affect the color as well as surface tension properties. LOMOLS were found to contain more phenolic hydroxyl-groups, more double bonds, and probably more carbonyl-groups. The major type of linkage seems to be one similar to a vinyl-polymer (two dimensional). This appears to be the result of the sulfonation of acid hydrolysis products of alkyl-aryl-ethers. Most properties of LOMOLS can be discussed in terms of this formation hypothesis.

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SECTION XII

ACKNOWLEDGEMENTS

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SECTION XIII

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SECTION XIV

PUBLICATIONS AND PATENTS

The following publications have been produced or are anticipated to be produced as a result of this project:

SPENT SULFITE LIQUOR XIV.	Preparation, Fractionation, and Preliminary Characterization of Cymnosperm Lignin Sulfonates from Spent Sulfite Liquor and Milled Wood Lignin. Forss, K., Collins, J.J., Glasser, W.G., Gratzl, J.S., and McCarthy, J.L., Tappi, 55, 1329-1333 (1972).
LIGNIN XVI.	Synthesis, Nuclear Magnetic Resonance, and Mass Spectroscopy of Several Monomeric and Dimeric Ligrin-like Sulfonates. Glasser, W.G., Gratzl, J.S., Collins, J.J., Forss, K., and McCarthy, J.L., Macromolecules, 6, 114 (1973)
LIGNIN XVII.	Preparation and Characterization of Acetyl Lignin Sulfonate Methyl Esters. Glasser, W.G., Gratzl, J.S., Collins, J.J., Forss, K., and McCarthy, J.L. To be submitted for publication in Macromolecules.

It appears that no patentable developments have arisen from the present study.

SECTION XV

GLOSSARY

ALSME - Acetylated lignin sulfonate methyl ester.

Ca/Na-SSL - Calcium or sodium base spent sulfite liquor.

DP - Degree of polymerization.

EIS - Ether insoluble.

GPC - Gel permeation chromatography.

HIMOLS - High molecular weight lignin sulfonates.

LOMOLS - Low Molecular Weight Lignin Sulfonates.

LS - Lignin Sulfonates.

MW - Molecular Weight

NMR - Nuclear magnetic resonance.

SSL - Spent sulfite liquor.

TLC - Thin layer chromatography

UV - Ultra violet spectroscopy.

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